A TOXICITY STUDY ON

"POONAGA PARPAM"

Dissertation Submitted To

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DOCTOR OF MEDICINE (SIDDHA)

(Branch – VI, Nanju Noolum Maruthuva Neethi Noolum)

Department of Nanju Noolum Maruthuva Neethi Noolum

Government Siddha Medical College

Palayamkottai – 627 002

OCTOBER – 2019

GOVT. SIDDHA MEDICAL COLLEGE, PALAYAMKOTTAI

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**A Toxicity Study on POONAGA PARPAM**" is a bonafide and genuine research work carried out by me under the guidance of **Dr. M. P. ABDUL KADER JEYLANI**, **M.D(s).,** Professor, Post Graduate Department of Nanju Noolum Maruthuva Neethi Noolum, Govt.Siddha Medical College, Palayamkottai, and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Signature of the Candidate**

Place: Palayamkottai **Dr. R. NITHYAMATHI**

CERTIFICATE

This is to certify that the dissertation entitled **"A TOXICITY STUDY ON POONAGA PARPAM"** is a bonafide work done by **Dr. R. NITHYAMATHI (Reg.No. 321616006)** Govt. Siddha Medical College, Palayamkotai in partial fulfillment of the university rules and regulations for award for **MD(s) Nanju Noolum Maruthuva Neethi Noolum** under my guidance and supervision during the academic year 2016-2019.

Name and signature of the Guide :

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This certificate is awarded to Dr/~~Mr/Mrs~~. R. NITHYAMATHI .....

for participating as ~~Resource Person~~ / Delegate in the XXIII Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

Organized by the Department of Siddha,

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CERTIFICATE OF MINERAL AUTHENTICITY

Certified the following the Jeevam drug used in Siddha formulation (Internal) **"POONAGA PARPAM"** for **"VINTHU KURAIVU "** taken up for Post-Graduation Dissertation Studies by **Dr.R.NITHYAMATHI,** PG Scholar of MD Siddha, Department of Toxicology, have selected the raw drug(Minerals) and have been authenticated through Geological methods(Macro/Micro).

S.NO	DRUG	ENGLISH NAME	SCIENTIFIC NAME
1	Poonagam	Earth worm	Lumbricus terrestris

Station : Palayamkottai

Date: 9-4-2018

Authorized Signature

Dr. M.I. ZAHIR HUSSAIN, M.Sc.,Ph.D.,
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TIRUNELVELI, TAMIL NADU

GOVERNMENT SIDDHA MEDICAL COLLEGE, PALAYAMKOTTAI

CERTIFICATE OF BOTANICAL AUTHENTICITY

Certified the following plants drugs used in Siddha formulation **"POONAGA PARPAM"** taken up for Post-Graduation Dissertation studies by **Dr.R.NITHYAMATHI** , PG Scholar MD Siddha, Department of Nanju Noolum Maruthuva Neethi Noolum, are correctly identified and authenticated through visual inspection / organoleptic characters / Experience and Training, Morphology, Microscopical and Taxonomical methods.

S. No.	Tamil Name (herbals)	Botanical Name	Family	Parts used
1	Pulippu Mathulai	Punica granatum	Punicaceae	Fruit

Station: Palayamkottai

Date: 20/2/19.

20/2/19

Authorized Signature

Dr. S. SUTHA, M.Sc.,M.Ed.,Ph.D.,
Associate Professor
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NANDHACOLLEGE OF PHARMACY, ERODE - 52

Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)
Institutional Animal Ethics Committee (IAEC)
Reg No: 688 /PO/Re/S/02/CPCSEA

CERTIFICATE

Title of the project	:	A TOXICITY STUDY ON POONAGA PARPAM
Proposal Number	:	NCP/IAEC/2018-19/23
Date received after modification (if any)	:	---
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Approval date	:	27.12.2018
Species & Number of animals sanctioned	:	WISTER ALBINO RAT/42
Expiry date (Termination of the Project)	:	05.04.2019
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PALAYAMKOTTAI

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This Certifies that

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has participated in Continuing Medical Education on "**AYUSH External Therapies-II**" held at GSMCH, Palayamkottai on Dec, 4 2018

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Head - Dept. of Sirappu Maruthuvam

Authorised Signatory
VAIDYARATNAM

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Editor in Chief

LIST OF TABLES

ABBREVIATIONS

PNP	POO NAGA PARPAM
No.	Number
Mg	Milligram
Kg	Kilogram
LD_{50}	Lethal $Dose_{50}$
ED_{50}	Effective $Dose_{50}$
p.o	peros
ML	Milliliter
%	percentage
R&D	Research and Development
EDTA	Ethylene Diamine Tetra Acetic Acid
M	Male
g%	Gram percentage
g	Gram
NOAEL	No-Observed-Adverse-Effect-Level
MLD	Minimum Lethal Dose
MTD	Maximum Tolerated Dose
OECD	Organization of Economic Co-operation and Development
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
FTIR	Fourier Transform – Infra Red Spectroscopy
SEM	Scanning Electron Microscopy
ICP-OES	Inductively Coupled Plasma Optical Emission-Spectrometry
LD	Low Dose
MD	Middle Dose
HD	High Dose
BDL	Below Detection Limit

1. INTRODUCTION

Siddha system of medicine is one of the ancient system of medicine practiced in India.."*Siddha*" derives from the word of "*Siddhar*" (Rishi) who formulated this therapy. Eighten Siddhars were said to have contributed towards the development of this medical system and Father of Siddha System is "LORD AGASTHIYAR" (MahaRishi).

According to siddhars concept the universe and livelihoods are made up of pancha poothas.

அண்டத்திலுள்ளதே பிண்டம்
பிண்டத்திலுள்ளதே அண்டம்
அண்டமும் பிண்டமும் ஒன்றே
அறிந்துதான் பார்க்கும் போதே

- சட்டைமுனி ஞானம்

In *sattai muni gnanam* human body is considered as microcosm and the universe as macrocosm, both microcosm and macrocosm were made up of panchapoothas. Siddha System is based on balancing of equilibrium of five Bhoothas (Doshas) as " LAND (*Prithvi*), WATER (*Appu)*, FIRE(*Theyu*), AIR (*Vayu*), SPACE (*Agayam)*.

Pancha poothas made the human body and vital forces namely vatham, pittham and kabam, they were also called as *uyir thathukkal*.

மிகினும் குறையினும் நோய்செய்யும் நூலோர்
வளிமுதலா எண்ணிய மூன்று

- திருவள்ளுவர்

In healthy human the three vital forces were in a balanced state , any imbalance in such forces may lead to diseases.

Any alteration in food habits, environmental and climatic changes will causes diseases.

மாறுபாடில்லாத உண்டிமறுத்து உண்ணின்
ஊறு பாடில்லை உயிர்க்கு

- திருவள்ளுவர்

Siddha system of medicine strictly advices food habits and its regulations as a rule along with the environmental and climatic changes.

உணவே மருந்து மருந்தே உணவு.

So, that the living beings will be free from disease, infections and help them to live longer healthy life. Siddhars who identified the nature's herbal medicinal value formulated the medicine on the basis of the *suvai , gunam,,veeriyam, vibhavam, prabhavam* of plants. Combined group of herbals to form a drug administrate in the infected.

Siddha system that insists that the physician should enquire the nature of the disease, its cause and its method of cure and treat it faithfully *Envagai Thaervugal* (the eight methods of clinical examination) are used to determine the diagnosis, aetiology, treatment and prognosis of diseases.

Siddhars are men with supernatural power and Medical wisdom. All their doing were highly intertwined with nature. They have combined their Medical works with astrology, alchemy & philosophy. They were robust in the concept "Nature that causes diseases and it is again nature effects their cure" and it is therefore necessary that the physician should know the principles of nature. Siddhars were proficient with formulations that treat and prevent ailments. The formulations were either purely herbal, animal, metals, a combination of herbomineral or metals. Thus for preparing medicine, Siddhars solely depend on natural wealth. Siddhars were persons of highly cultured intellectual & spiritual faculties with a very vast boundless knowledge about medicine and alchemy.

Drug means any substance that when taken into a living organism may modify physiological system or pathological states.

A drug possessing high therapeutic index is said to be safe. Reaction to a drug in a subject may vary in accordance with various factors which includes anaphylaxsis, idiosyncrasy or accidental overdosing. Variation of dose, duration alters the medicine and makes it a poison.

Dose and duration of administration of drug is much more important for clinical practice. It is the duty of a physician to ensure that the patient is receiving the correct dose and is harmless. Or else, the physician will be charged for his medical negligence under legal aspects.

Toxicology is the science of poison, an evolving discipline, moving from art to science from using chemicals which hurt to chemicals that prevent hurting, from taking lives to saving lives. It is the aspect of Pharmacology that deals with adverse effects of drugs. It is concerned not only with drug used in therapy but also with the many other chemicals that may be responsible for house hold or environmental,

industrial intoxication. The toxic effects of any chemical is such an extensive subject that the clinician must pay attention to it, to prevent, recognize, treat those deleterious effects.

Our Siddhars paid more attention to herbs and also jeevam as they were used in curing diseases and also in alchemy. Hence I select “Poonaga Parpam” is mainly used in Spermatogenesis activity. Evaluation on safety aspects using scientific parameters is essential to cope up with the growing scientific world. Poonaga Parpam is commonly used in Male infertility like Impotence, Oligospermia etc.

A scientific evaluation is the need of the hour for this common medicine which is used extensively. This study will provide a scientific evidence for the safety of “Poonaga Parpam” to be used clinically.

2. AIM AND OBJECTIVES

AIM:

The main aim of this study is to access the safety of the drug "POONAGA PARPAM" on wistar albino rats under various dose levels of drug administration especially in acute and sub acute toxicity studies.

OBJECTIVE:

- To collect the literature and other evidences of each ingredient on pharmacological and toxicological aspect.
- To collect and purify the raw drugs according to literature evidence.
- To prepare the medicine based on the procedure quoted in literature.
- To establish the acute and sub acute toxicity of the drug.
- To evaluate the biochemical analysis of the drug.
- To analysis the haematological investigations and histopathological study of the organs such as kidney, liver, heart and brain in wister rats.
- To create an awareness among the practitioners of siddha to go for further study regarding the adverse effect in the drug.

3.REVIEW OF LITERATURE

SIDDHA ASPECT

POONAGAM

NAME:

Poonagam

ZOOLOGICAL NAME:

Lumbricus terrestris

OTHER NAME:

மேகத்தின் விந்து விளங்கு சஞ்சீவிதான்
பாசத்தின் சோதி பருவிய னங்களு
நாகத்தின் பாம்பு நலமான பூமி வேள்
போச ரசதீபன் பூநாகப் பேருமே

The other names of earth warm *megathin vinthu, sanjeevi, sothimayamana vethi,mangulathan,poomi vear,aagasatheepan.*

- சட்டை முனி நிகண்டு

Nakku poochi, poomi vear, naangool puzhu, mansumantha vasugi, kandapatham

- குணப்பாடம் தாது சீவ வகுப்பு

"பூநாகம் தானன்றும் பூறைக் காலி
பெருத்தநில வேரென்றும் பூசி பானென்றும்
கூநாகம் கோபனு ரத்த ஐந்து
குளமான பூலதாசந் தாகஞ் சித்தி
நாநாகம் மாதத்துண்ட கமுகாகுந்
நன்மையான வச்சிர நல் மணியுமாகும்
வேநாகம் விடங்களெல்லாம் போக்கி வைக்கும்
விடுபட்ட பூநாக விவரமாமே''.

- போகர் நிகண்டு

Naangoozh, poonagam, poomivear, megathin vinthu, pooraikaali, nilaveer, poosipan, ratha senthu, pooladhasam, nanagam, thundagam, purudotha senthu

PREPARATION OF EARTH WORM:

Dig the gravel land at one feet inside the hollow the millet husk should be placed. Then spit the water on the husk. Later cover the hollow with mat and coconut sheath, the water should be spit everyday at one time it should be continued for 10 days or one week. Finally the earthworm will be produced.

TYPES OF EARTH WORM:

உபித்திடவே பூநாகம் விதம் ரெண்டாகும்
உண்மையாக கருதி போலொன்றுண்டு தானே
ஒன்றுதானே வெளிறின தோர் சிவப்புமாகும்
உயர்ந்திட்ட சிவப்பது தான் உத்த மந்தான்
பன்று தானே சத்துக்குப் பலித மொத்த
பாங்கான ரசாயனத் தீபனியு மாகும்
வின்று தானே வெளிறின தோர் சிவப்பு தானும்
மேன்மையான மருந்துக்கு நிதியுமாகும்.

Earthworm was obtained from marshland. It is of two types. The bright red colored was used for chemical purposes and the one which was pale red in color was used for preparing medicines. Of the two types, bright red colored was the best. They contain copper content.

PURIFICATION OF EARTH WORM:

ஆமென்ற முன் சொன்ன பூநாகந்தான்
ஆளந்து மேதான படியொன்று பாலில்போட
வாமென்ற மண்ணெல்லாங் கக்குங் கீழே
மறுநாள் தான் அதையெடுத்துச் சட்டியிலிட்டு
நேமென்ற நீர் விட்டுக் கழுவி போட்டு.

The poonagam were put into cow's milk. Let the poonagam to drink the milk and to vomit out the mud ingested by them then they were taken out and washed in the running water.

GENERAL CHARACTERISTICS:

மாதவறு செய்வறட்சி மாறுமடங் காச்சந்தி
பாதவறு நோயோடு பாறுமடல் வாதவாறு
குண்டபதமீ; மேக்கான மையமும் போங்
கண்ட பத மென்றுங் கால்.

Poonagathal migavum thunbathai vilaivikinra thaga rogam (bilious diseases*), Ezhu vitha asathiya sannipatha surangalum pogam (* Typhus Fever *). Oorusthamba vatham (*numbness of thigh*) engira mahavathamagiya puliyinal kavarnthu kollapatta thodaiagiya uruppu meezhuvathodu okkalamum kaphanoiyum olyum enga.*

USES OF EARTH WORM:

- Purified earth worm is dried and powdered and taken about 12.6g with grape juice for kidney stone.
- Earth worm powder is mixed with Terminalia cattapa, ghee and it is applied for parturition pain, hydrocoele.
- Earthworm is boiled with gingely oil and taken for chronic cough and throat pain.
- Earthworm taken with meat soup increases the aphrodisiac activity.
- Earth worm and dried leech is taken in equal ratio boiled in gingelly oil and it is applied on male external genitalia to increases aphrodisiac activity.
- Earthworm is used externally for cut and bleeding wounds. Earthworm and black stone is made into paste and applied for joint dislocation and induces inflammation.

பூநாக செந்தூரம்:

மோரிலிட்டு சுத்தி செய்த பூநாகத்தை ஆடுதீண்டாப்பாளைச் சாறு விட்டு அரைத்து 10 புடமிட்டெடுக்க செந்தூரம் ஆகும்.

இதனை குன்றி அளவில் தக்க துணைமருந்தில் கொடுத்து வர சுரம் குணமாகும்.

பூநாக கருக்கு குடிநீர்:

பூநாகம் 35 கிராம், மயிலிறகு 8.75 கிராம் இவை இரண்டையும் புதுச் சட்டியிலிட்டுக் கருக்கி, அதில் சிறிதளவு தேன் விட்டு 500 மிலி நீர் சேர்த்து அதனுடன் ஏலக்காய் தோல் 8.75 கிராம் கூட்டி நாலில் ஒன்றாய்க் குறுக்கிக் காலை, மாலை கொடுத்து வர நாவறட்சி, சந்நிதோடம் நீங்கும்.

பூநாக கருக்கு:

பூநாகம், மயிலிறகு இரண்டையும் சமனெடை எடுத்து சட்டியிலிட்டு சாம்பலாக்கி 650 மிகி தேனில் குழைத்து நாவில் காலை மாலை தடவி வர நாவறட்சி, சந்நிதோடம் நீங்கும்.

பூநாகச் சத்து:

நேர்ந்தது வேர் ஆட்டுச் சானிதள்ளி லேதான்
வேமென்ற பூநாகம் தனைப் பிசைந்து
வில்லை போலே இலேசாகப் பண்ணிடாயே

பண்ணியுமே மிடுக்கான வெயிலில் போட்டு
பாந்தமாக வுலர்த்தியே தான் மன் விழாமல்
புண்ணியமே பெரும் பாண்டத் தனில வைத்து
பெருஞ் சட்டியால் மூடி மண்ணைச் செய்து
நண்ணியுமே பால் சாமம் எரித்த பின்பு
கருதியதோர் சாம்பலெல்லாம் கழலவூவதோ
கழலவேதான் சாம்பலெல்லாங் கரைத்தி றுத்து
கனமான செம்பெல்லா மடியில் நிற்கும்
மழல வேதான் மேல் சாம்பல் இறுத்துப் போட்டு
மின்னலாக செம்பு போல யடியில் நிற்கும்
கழலவேதான் சூதமொரு பலந்தான் போட்டு
துழாவிடவே செம்பெல்லாம் பிடித்துக் கொள்ளும்
உழலவே தான் உருண்டை போல் வாங்கிக் கொண்டு
ஒக்கவே தான்போடு கிறமருந்தைக் கேளே

-போகர் 7000 (மூன்றாமாயிரம்)

பாலில் சுத்தி செய்த 1 படி (2லிட்டர்) பூநாகத்தை மேஷச் (ஆட்டு) சாணியிலிட்டுப் பிசைந்து, சிறு வில்லைகளாய்ச் செய்து, வெயிலில் உலர்த்திப் பாண்டத்திலடைத்துச் சட்டி கொண்டு மூடிச் சீலை மண் செய்து 12 மணி நேரம் எரித்து ஆற விட்டுக் காடி வார்த்து கையால் பிசைந்து கரைத்து சாம்பல் நீரை இறுத்து விட்டால் செம்பின் சத்து அடியில் தங்கும். இரசம் 35 கிராம் போட்டு துழாவ செம்பு பற்றி உருண்டையாகும். இதனைக் குகையிலிட்டு வெங்காரம் கொடுத்து உருக்க இரசம் பரிணமித்து விடும். செம்பு மாத்திரம் உருகித் தங்கும். இதனை செந்தூரம் முதலியனவாகச் செய்து உபயோகிக்கலாம்.

-குணபாடம் தாது ஜீவ வகுப்பு.

பூநாகச் சூரணம்:

பூநாகம்	- 1 பலம்
தூதுவளை சமூலம்	- 1 பலம்
திரிகடுகு	- ¼ பலம் (வகைக்கு)
திரிபலா	- 1/8 பலம் (வகைக்கு)
சிற்றரத்தை	- 1/8 பலம்
கற்கடகசிங்கி	- 1/8 பலம்
மூங்கிலுப்பு	- 1/8 பலம்
சீந்தில் கொடி	- 1/2 பலம்

எடுத்து உலர்த்தி சூரணித்து சம எடை சீனாக்கற்கண்டு பொடி கலந்து எடுத்து பத்திரப்படுத்தவும்.

அளவு : 1-2 கிராம்

தீரும்நோய் : காசம்,சயம், குன்மம், இரத்தகாசம், சூதகவாயு, மேக உட்ணம் தீரும்.

- சரபேந்திரர் சித்த மருத்துவச் சுடர்.

பூநாக பற்பம்:

நாகர வண்டு ஓடு 6 பலத்திற்கு சக்திச்சாரணை சாறு விட்டு நன்கு அரைத்து மூசையும் மூடியும் செய்துலர்த்தி அதில் சுத்தி செய்த பூநாகம் 3 பலமிட்டு மூடி ஒரு சட்டிக்குள் வைத்து மேல் மூடி சீலை மண் செய்து 4 சாமம் எரிக்க பற்பமாகும்.

அளவு : பணவெடை வீதம்

துணைமருந்து : திரிகடுகு, தேன்

தீரும்நோய் : சுரம், சந்நி தீரும்.

- தேரையர் வைத்தியம்.

பூநாக பற்பம்:

ஒரு வீச்சை சிவந்த பூநாகம் கொண்டு வந்து மோரில் போட்டு மண்ணெல்லாம் கக்கின பின்பு சுத்தமாக அலம்பிக் காயவைத்து இடித்துக் கல்வத்திலிட்டு ஆடுதீண்டாபாளைச் சாறு விட்டு ஒருநாள் அரைத்து வில்லை செய்து வெய்யிலில் உலர்த்தி அகலில் இட்டு சீலை செய்து 100 வரட்டியில் புடமிட பற்பமாகும்.

அளவு : வேளைக்கு ½ முதல் 1 குன்றி எடை இருவேளை 15 நாள்

துணைமருந்து : நெய், தேன்

தீரும்நோய் : கொடிய காசம், மேகசுரம் தீரும்.

பத்தியம் : சமயத்துக்கேற்ப அறிந்து பத்தியமிருத்தல் நலம்.

- சிகிச்சாரத்ன தீபம்

பூநாகத்தினால் தாகம், சந்நிபாதம், ஊருஸ்தம்பவாதம், வலி, இசிவு, கபநோய்கள், குணமாகும். பூநாகத்துடன் சிறிது ஏலரிசி சேர்த்தரைத்து எடுத்த கற்கம் பாக்களவு வீதம் கொடுத்து வர வலி இசிவு தீரும்.

இத்துடன் நொச்சியிலை சேர்த்தரைத்து நல்லெண்ணயில் கலந்து தைல பதமாக காய்ச்சி குடித் தைலமாக வழங்க மண்டை குடைச்சல், கழுத்து நரம்புகள் இசிவு, சீதளத்தினால் ஏற்பட்ட தலைவலி, தலைபாரம் தீரும்.

-அனுபவ வைத்திய தேவரகசியம் (முதல் பாகம்)

அயத்தங்கச் செந்தூரம்:

அயம் -17 ½ கிராம்

தங்கம் -17 ½ கிராம்

பூநாகம் -17 ½ கிராம்

இவற்றைப் பேய்க் கரும்புச் சாற்றில் நெகிழ அரைத்து வில்லை தட்டி வெயிலில் காயவைத்து அகலில் வைத்து மேல் மூடி சீலை மண் செய்து ஒரு பெரிய

சட்டிக்குள் பாதியளவு உப்புக் கொட்டி அதன் மேல் அகலை வைத்து மேலும் உப்புக் கொட்டி மேல் சட்டிக் கொண்டு மூடிச் சீலை செய்து 12 மணி நேரம் முத்தீயிட்டு எரிக்க உயர்ந்த செந்தூரம் ஆகும்.

அளவு : குன்றி எடை இருவேளை

துணைமருந்து : தேன், நெய்

தீரும் நோய்கள்: குளிர்சுரம், நீரிழிவு

- சரபேந்திரர் சித்த மருத்துவச் சுடர்.

பூநாக பற்பம்:

சுத்தித்த பூநாகம் தேவையான அளவு எடுத்து ஒரு கலயத்துள் போட்டு மேல் அகல் மூடி சீலை செய்து வெய்யிலில் உலர்த்தி கலசத்தின் 20 பங்கு எடை வறட்டியில் புடமிட்டு ஆறிய பின் எடுக்க பற்பமாகும். இதனை எடுத்து பொடித்துத் துணியில் வடிகட்டி பளிங்கு குப்பியில் பத்திரப்படுத்தவும்.

அளவு : 2-4 குன்றி எடை

துணைமருந்து : தேன், நெய், வெண்ணெய், சர்க்கரை, நீர்.

தீரும்நோய்கள் : கபநோய்கள் குணமாகும். தளர்ந்த நரம்புகள் முறுக்கேறி பலப்படும். விந்து வெளிப்பாடு நீங்கும்.

- அனுபோக வைத்திய நவநீதம் 8ம் பாகம்.

நாட்பட்ட இருமல் தீர:

எண்ணெறயிற் பூநாக மெரித்திட்டிட நாட்படுமிருமல்

குண்ணதிற் போல குறுகியது

நல்லெண்ணெயில் பூநாகத்தை சிதைத்துப் போட்டு காய்ச்சி பதத்தில் இறக்கி வைத்து வடிகட்டி இறுத்து வைத்து அதில் கரண்டி வீதம் 3,5,7 நாட்கள் குடித்து வர நாட்பட்ட இருமல், இரைப்பு முதலியன நீங்கும்.

- வைத்திய பெருங்குறள் -828

பூநாக செயநீர்:

இந்துப்பை பூநாகத்திற் றூவ செயநீராய்

வந்து பயங் கரைக்கு மதில்

நாக்குபூச்சியை ஒரு பாத்திரத்தில் போட்டு அதன் மேல் கொஞ்சம் இந்துப்பை தூவி விடவும். அது தண்ணீராய் கரைந்து செயநீராகி விடும்.

மேற்படி செயநீர் ¼ மாகாண படி, வேப்பெண்ணெய் ¼ மாகாண படி, மூட்டுத் திரி மூன்று எடுத்து எடுத்து இவற்றை நன்கு காய்ச்சி பதத்தில் இறக்கவும். வாரம் ஒரு முறை கொடுக்க இளைப்பு இருமல் தீரும்

பூநாக கருக்குகியாழம்

தீரும் நோய்கள் : வலிப்பு, உடல் வறட்சி

அளவு : 30மி

- பதார்த்த குணவிளக்கம்

நாயுருவி கருக்கு கியாழம்:

தீரும் நோய்கள் : அனைத்து வகையான உடல் வறட்சி

அளவு : 30 மி

- கியாழ இலம்பகம்

பூநாக மேற்பூச்சு தைலம்

உபயோகம் : வெற்றிலையில் தடவி பாதிக்கப்பட்ட இடத்தில் பற்றுபோட வேண்டும்.

தீரும் நோய்கள் : நரம்பு தளர்ச்சி, உடல்வலிவு

- அனுபோக வைத்திய நவநீதம்

பூநாக தைலம்

தீரும் நோய்கள் : நரம்பு தளர்ச்சி, உடல்வலிவு

அளவு : 5 – 10 துளிகள்

- அனுபோக வைத்திய நவநீதம், பாகம்-3

பூநாக திலா

தீரும் நோய்கள் : நரம்பு உரமாக்கி

அளவு : 520 மிகி

- அனுபோக வைத்திய நவநீதம், பாகம்-3

மன்மதலோக செந்தூரம்

தீரும் நோய்கள் : உடல்வன்மை, உடல் சக்தி, நரம்பு உரமாக்கி

அளவு : 175 – 210 கிராம்

- அனுபோக வைத்திய நவநீதம், பாகம்-1

நாவறட்சி கியாழம்

தீரும் நோய்கள் : தாகம், விக்கல், நாவறட்சி, நாவில் சுரசுரப்பு

அளவு : 30மி

- *சிகிட்சா ரத்ன தீபம்*

பூநாக சூரணம்

தீரும் நோய்கள் : தாகம், மேகசூலை, சயம், சோபை

அளவு : 2.9 கி

- *சிகிட்சா ரத்ன தீபம்*

புளிப்பு மாதுளை

Tamil name	:	புளிப்பு மாதுளை
Botanical name	:	Purica granatum.Linn
வேறுபெயர்	:	தாடிமம், பீசபுரம், மாதுளங்கம், மாதுளம், மாதுளுங்கம், கழுமுள்
Eng	:	Pomegranate
Tel	:	Danimna
Arab	:	Rumaman
Mal	:	Mathlam
Pers	:	Gulmarn
Kan	:	Dalimba
Hin	:	Anae
Sans	:	Shukhdana
Dak	:	Darim

மாதுளை

இது சிறு மரவகுப்பைச் சேர்ந்தது. முக்கியமாய் ஆப்கானிஸ்தான், பெலுசிஸ்தான், பாரசீகம் முதலிய இடங்களில் அளவுகடந்து வளருகின்றது. இந்தியாவில் எல்லாவிடங்களிலும் பயிரிடப்பட்டு வருகிறது. (இதில் இனிப்பு மாதளை, புளிப்பு மாதளை, பூமாதளை என்று மூன்று வகுப்புகளுண்டு.)

பயன்படும் உறுப்பு:

பூ, பிஞ்சு, பழம், விதை, பட்டை, பழத்தோல்.

ORGANOLEPTIC CHARACTERS

பட்டை

சுவை (Taste)	-	துவர்ப்பு (Astringent)
தன்மை (Potency)	-	தட்பம் (Coolent)
பிரிவு (Bio transformation)	-	கார்ப்பு (Pungent)

பழம் விதை

சுவை (Taste)	-	இனிப்பு (Sweet)
தன்மை (Potency)	-	தட்பம் (Coolent)
பிரிவு (Bio transformation)	-	இனிப்பு (Sweet)

செய்கை

Astringent	-	துவர்ப்பி	-	ஸங்கோசனகாரி
Styptic	-	குருதிப்பெருக்கடக்கி	-	ரத்தஸ்தம்பனகாரி

பூ, பழத்தோல்

Astringent	-	துவர்ப்பி	-	ஸங்கோசனகாரி
Stomachic	-	பசித்தீத்தூண்டி	-	ஜடராக்னிவர்த்தினி

மரப்பட்டை வேர்ப்பட்டை

Anthelmintic	-	புழுக்கொல்லி	-	கிருமிநாசினி

பழம்

Refrigerant	-	குளிர்ச்சியுண்டாக்கி	-	சீதளகாரி

விதை

Astringent	-	துவர்ப்பி	-	ஸங்கோசனகாரி
Anthelmintic	-	புழுக்கொல்லி	-	கிருமிநாசினி
Toenifuge	-	சாயமேற்றி		
Aphrodisiac	-	ஆண்மைபெருக்கி		
வீரியம்	-	தண்மை (குளிர்ச்சி)		

தண்மை வீரியத்தின் தொழில்

மனக்களிப்பு, ஆயுள்விருத்தி, தம்பனம் ஆகிய காரியங்களைச் செய்யும். இரத்தம் பித்தம் ஆகியவற்றைப் போக்கும்.

T.V. சாம்பசிவம் பிள்ளை அவர்கள் மாதளையின் வகைகளைப் பற்றி பின்வருமாறு கூறியுள்ளார்.

(T.V.S. அகராதி பாகம் - 5 ப.எண்.906)

1. பூ மாதளை	-	A male variety of pomegranate
2. புளிப்பு மாதளை	-	Which beals only soul fruits; fruits useful in vomiting.
3. தித்திப்பு மாதளை	-	Which bears only sweet fruits, fruits are used as tonic.
4. கொடி மாதளை (சீதளங்காய்)	-	A variety of citrus.
5. கொம்மட்டி மாதளை	-	A variety of citrus.
6. தாது மாதளை, same as பூ மாதளை	-	Punica granatum
7. வயல் மாதளை		
8. வெள்ளை மாதளை	-	One with white pulpy seeds
9. சீமை மாதளை	-	Cynodia vulagaris

10. தாடி மாதளை - Punica Granatum

11. 11. சிவப்பு இரட்டை மாதளை - A male variety of pomegranate

மாதுளை பூ

குணம்

இதனால் குருதிவாந்தி, வயிற்றுக்கடுப்பு, வெப்பம், குருதிமூலம் இவை போகும். இது குருதியை பெருக்கும். வன்மையைத் தரும்.

"வாந்திபித்த தோடமொடு மாறாக் கடுப்பனலஞ்
சேர்ந்துநின்ற மூலரத்தந் தீர்க்குங்காண் - மாந்தளிர்க்கை
மாதே! யிரத்தபுஷ்டி வல்லபலன் உண்டாகும்
பூதலத்துள் மாதளையின்பூ"

பிஞ்சு

இதனால் கழிச்சல் வகைகள் யாவும் போகும்.

"மலக்கழிச்சல் சீதத்தால் வந்த கழிச்சல்
சலக்கழிச்சல் சோரியாற் காரும் - பலக்கழிச்சல்
மாளும் புளிப்பான மாதுளம் பிஞ்சையுண்ண
ஆளும்கண் மாதே ஆறி"

பழம்

"மாதுளைக் கனியுண மதனகா மேசுரத்
சூதென வாயுளர் சொல்லுவர் மிக்கவே".

பொருள்:

மாதுளம் பழத்தைத் தினமும் புசித்துவரின், ஆண்மைப் பெருக்கு முதலியன உண்டாகும்.

இப்பழத்தினால் முப்பிணி சுரத்தில் காண்கின்ற வாந்தி, நீர்வேட்கை, இவைகள் போகும். பிள்ளை உண்டாகாமற்படி செய்கின்ற சூலகநோயைப் போக்கும். அன்றியும் வாய்நீர் ஊறல், விக்கல், மந்தம், வெப்பத்தால் உண்டான காய்ச்சல் நெஞ்செரிவு, காதடைப்பு, மயக்கம் இவை போகும். மேலும், உடல் குளிர்ச்சியடையும் மேற்கூறிய பண்புகளைப் பெறத் தானாகப் பழுத்து வெடித்த மாதுளையின் முத்துகளை எடுத்துத் துணியிலிட்டு பிசைந்து சாறெடுத்து வேண்டிய அளவு கற்கண்டு கூட்டி உடனே குடித்தல் வேண்டும்.

"சங்கையறச் சொற்றவிர்க்குஞ் சன்னியா சஞ்சர்த்தி
யுங்கையதி காதமைச் சாருமோ – கங்கை
இருந்தாடி மக்கட் கிரத்தலைச்செய் நோய்போம்
இருந்தாடி மக்கனிகட் கெண்"

“வாய்நீரு றல்கசப்பு வாந்திவிக்கல் மந்தமிக்க
காய்வெப்பம் நெஞ்செரிவு காதடைப்பும் - ஓயா
மயக்குமுந் தீர்ந்துவிடும் மாதுளம் பழத்தால்
தயக்கமறத் தேமொழியே காற்று”.

பூவை உலர்த்திச் சூரணித்து அதில் 4கி எடுத்து வேலம் பிசின் தூள் 4கி, அபின் 195மிகி சேர்த்து, வேளைக்கு 260-390 வீதம் கொடுத்து வர சீதக்கழிச்சல், குருதிக்கழிச்சல், குருதிநீர், இரத்தமூலம் இவை நீங்கும்.

பிஞ்சைக் குடிநீரிட்டு சீதபேதி, அதிசாரம் முதலியவைகளுக்கும் கொடுப்பது நாட்டு வழக்கம்.

(வ.கு): இதன் பழச்சாற்றில் கற்கண்டு சேர்த்து மணப்பாகு செய்து அருந்த, அழலைப் போக்கும். குளிர்ச்சியை உண்டு பண்ணும். சுரம், அழல், தாகம் இவை நீங்கும்.

- பழச்சாற்றை இளைப்பு நோயினர்க்குக் கொடுக்க, மிகுந்த நன்மை தரும். குணத்தின் கீழ்க்கண்ட நோய்களை விலக்கும்.
- பழத்தோலுடன் சிறிது இலவங்கம், இலவங்கப்பட்டை, நசுக்கி போட்டு முறைப்படி குடிநீரிட்டு 15-30 மிலி வீதம் தினம் 3-4 வேளை கொடுத்து வர நாட்பட்ட சீதக்கழிச்சல் நீங்கும். பழத்தோல், மங்குஸ்தான் பழத்தோல் பாலை மரப்பட்டை வகைக்கு 40கி எடுத்து, 1400 மிலி நீர் விட்டு முறைப்படி குடிநீரிட்டு அதில் 15-30 மிலி கொடுக்கலாம்.
- மேலும் பலவிதக் கழிச்சல்களுக்கு துவர்ப்பு மருந்து வேண்டியதாயிருக்கும் போது இதை கொள்ளலாம்.
- பழத்தோலை உலர்த்திப் பொடியாக்கி வழங்கலாம்.
- பழத்தோல் பொடி 17 கி வெள்ளைப் போளம் 17கி, சீமைச் சுண்ணாம்புத்தூள் 34கி சேர்த்து கலந்து பல்துலக்க, பல்வலி போகும்.
- வேர்ப்பட்டை ஒருபங்கு, நீர் இருபது பங்கு விட்டு சிறிது இலவங்கம் சேர்த்து, எட்டில் ஒன்றாக காய்ச்சிக் கொடுக்க, தட்டைப்புழு விழும்.

“வெடித்துவீழ் பழத்தை வாங்கி மெல்லிய சீலை கட்டி
கடுக்கெனப் பிழிந்து கொண்டு கண்டகர்க் கரையுங் கூட்டிக்
குடித்திட வெடிப்பு மாறுங் குளிர்ந்திடும் அங்கமெல்லாம்
வடித்ததநன் மொழியி னாளே மாதுளம் பழத்தின்சாறே”

வ.கு. பூ மொக்கை உலர்த்திப் பொடித்து – 130மிகி கொடுக்க இருமல் நீங்கும்.

மாதுளம் பிஞ்சுக் குடிநீர்

"முகமா துளைப்பிஞ் சதிவிடயம் முத்தக் காசு பெருமரத்தோல்
தகைசேர் சுக்கு விளங்காயின் சதையோ ரொன்று கழஞ்சிரண்டு
வகையாய்க் கூட்டி யிருநாழி யுழக்காய்க் காய்ச்சிப் போதருந்ந
பகையாய் வருமதிசாரசுரம் போகும் பாரிற் பாரீரே"

பொருள்

மாதுளம் பிஞ்சு, அதிவிடயம், முத்தக்காசு, பெருமரத்தோல், சுக்கு, விளாங்காய்த்தசை இவை ஒவ்வொன்றிலும் இரண்டிரண்டு கழஞ்சுகளெடுத்து ஒன்று சேர்த்து, இரண்டு நாழி நீர் விட்டு அதை உழக்காய்க் குறுக்கிக் குடிக்க, கழிச்சல், சுரம் பறந்து போகும்.

மாதுளம் பிஞ்சு

- மேற்படி பொடியுடன் ஏலக்காய்த்தூள், கசகசாத்தூள், குங்கிகலியத்தூள் ஓரெடை சேர்த்து, 65மி.கி அளவு தினம் இருவேளை கொடுத்து வர நாட்பட்ட பெருங்கழிச்சல், சீதக்கழிச்சல் இவை நீங்கும்.
- பூவின் சாறும் அறுகம்புல்லின் சாறும் ஓரெடை சேர்த்துக் கொடுக்க மூக்கிலிருந்து குருதி வடிவது நீங்கும்.
- மேற்கூறிய பூவையும் புல்லையும் குடிநீரிட்டு பொரித்த வெங்காரம் சிறகு கூட்டி, வாய் கொப்புளித்து வரத் தொண்டைநோய் நீங்கும். இதனை 15-30மிலி வீதம் காலையில் வெறும் வயிற்றில் இரண்டு மணிக்கொரு தடவையாக ஆறுதடவை குடித்துக் கொண்டே வந்து, கடைசித் தடவையில் கழிச்சலை உண்டாக்கும் மருந்துகளைக் கொடுக்க புழு வெளியாகும்.
- மேற்படி குடிநீரைக் குழந்தைகளுக்குண்டாகும் இருமல் நோய், கண்நோய், பெரியவர்களுக்குண்டாகும் நாட்பட்ட சுரம், முறைச்சுரம், மண்ணீரல் தாபிதம் இவைகளினாலுண்டாகும் குருதியழல் முதலிய நோய்களுக்கும் வழங்கலாம்.

உபயோகம் (அனுபோக வைத்திய பிரம்மரகசியம் 2-ம்பாகம் ப.எண்.263)

ஒரு மாதுளம் பிஞ்சைக்கொண்டு வந்து மேல்தோல் நீக்கி அதின் உள்ளிருக்கும் விதையை சாப்பிட்டு வர அதிசாரம் (9) ம் தீரும். ஆனால் 10 நாளைக்கு சாப்பிட வேண்டும். மேற்சொன்ன பிரகாரம் பழத்தைச் சாப்பிட பித்தம், தாகம், கபம், வாந்தி இவைகள் தீரும்.

மாதுளங்குடிநீர்

"மாதுளங் கடம்பு புன்கின் வாய்த்ததேவர் திப்பிலி யத்தித்
தீதறுங் கொழுந்து சுக்குதிரிபலைவி கும்பிக் கூட்டி
நீதியா யிரண்டு நாழி நீருழக் காக்கிக் கொண்டால்
சூதினை முலையினாளே சுரமதி சாரம் போமே"

பொருள்

மாதுளம், கடம்பு, புன்கு இவற்றின் வேர்கள், திப்பிலி, அத்திக்கொழுந்து, சுக்கு, திரிபலை ஆகிய இவை ஒவ்வொன்றையும் ஒரே எடையில் சேர்த்து 4 லிட்டர் நீர்விட்டு அதை 1 லிட்டராகச் சுண்ட வைத்து அருந்தில், சுரக்கழிச்சல் தீரும் (தேரன்-நூறு)

மாதுளம்பட்டை

பட்டைச்சாறு 17 கிராம் கொடுக்க, அது வயிற்றுப் புழுவைக் கொல்லும்.

விதை

விதையானது நீர்த்துப்போன வெந்நீரை இறுக்கும். வெள்ளையில் காணும் நீர் கடுப்பை நீக்கும். இவ்விதை, உடற்கு ஊட்டத்தையும் ஆண்மையையும் உண்டாக்கும் பலவித இலேகியங்களில் சேரும்.

“சுக்கிலத்தை கட்டுமந்தச் சுக்கிலதோ டம்போக்கும்
அக்கணமே கக்கடுப்பை ஆற்றுங்காண – எக்காலுங்
கோதுனத்தெண் ணாதமலர்க் கொம்பளைய மாதேசெம்
மாதுளத்தின் பீச வலி”

மாதுளை மரத்தின் வேர் பிஞ்சு

மாதுளை வேர், பிஞ்சு இகைளினால் வாந்தி, அதிசாரம் நீங்கும். பழத்தினால் தாது, ஆண்மை விருத்தி உண்டாகும். தாகம் போகும். இலை, காய், மலர் இவைகளினால் முற்கூறிய நோய்கள் யாவும் விலகும்.

“மாதுளைவேர் பிஞ்சிவைக்கு வாந்தியதி சுரம்போம்
தாதுவுமாம் அக்கினிக்குத் தாகம்போம் - சூதைநிகர்
வன்னமுலை யாய் இலைகாய் மாமலர்க ளுக்குமுனஞ:
சொன்னவை யெலாம் போகுஞ் சொல்”

மாதுளம்பழத்தின் ஓடு (Punica Granatum – Dried rind)

“சீராக்கும் வாய்ப்புண்ணைச் சீதமுடன் ரத்தமெனப்
பேராக்கும் பேதிகளைப் பேசுங்கால் - நேராக்கும்
தாதுமே லோங்கும் தனியாத தாகமறும்
மாதுளத்தின் நற்கனித்தோல் மாண்பு”

குணம்:

மாதுளம்பழத்தின் மேற்றோலினால் வாய்ப்புண் சீதபேதி, ரத்தபேதி முதலியவைகள் போம். தாது பலப்படும் என்க.

செய்கை

சங்கோசனகாரி, வியதாபேதகாரி

உபயோகிக்கும் முறை

உலர்ந்த மாதுளம்பழத்தின் தோலை இடித்து, 2 ½ ரூபாய் எடை சூரணத்தை ஒரு பழகின மட்குடுவையில் போட்டு ½ படி சலம் விட்டு, 15-20 நிமிஷம் கொதிக்க வைத்து வடிகட்டி வைத்துக்கொண்டு வேளைக்கு 1-1 ½ அவுன்ஸ் வீதம் தினம் 3 வேளை உள்ளுக்குக் கொடுத்து வரச் சீதபேதி, ரத்தபேதி முதலியவைகள் போகும். இந்தக் கியாழத்தைக் கொண்டு வாய் கொப்பளித்து வர வாய்ப்புண், மருந்துகளின் வீறினாலுண்டான வாய் வேக்காடு முதலியவைகள் குணமாகும்.

மாதுளம் பிஞ்சு

"மலக்கழிச்சல் சீதத்தால் வந்த கழிச்சல்
சலக்கழிச்சல் சோரியாற் சாரும் - பலகழிச்சல்
மாளும் புளிப்பான மாதுளம்பிஞ் சாலயிலை
யாளுங்கண் மாதே யறி".

குணம்

புளிமாதுளம் பிஞ்சால் மலம், சீதம், சலம், ரத்தம் இவைகளினிறமாக ஆகின்ற பற்பல **அதிசார ரோகங்கள் தீரும்.**

செய்கை

சங்கோசனகாரி, வியதாபேதகாரி

உபயோகிக்கும் முறை

புளிப்புச் சுவையையுடைய மாதுளம் பிஞ்சை உலர்த்தி அதன்மேல் தோலை இடித்துச் சூரணம் செய்து வைத்துக் கொண்டு, வேளைக்கு 10-15 குன்றி எடை தயிரில் கலக்கிக் கொடுக்க சீதபேதி, ரத்தபேதி, நிவர்த்தியாகும். இதன் பிஞ்சுக்காயை அரைத்து ஒரு சிறு எலுமிச்சங்காய்ப் பிரமாணம் தயிரில் கலக்கிக் கொடுக்கச் சீதபேதி, ரத்தபேதி முதலியவைகள் குணமாகும். புளிப்பு மாதுளம் பிஞ்சு கிடைக்காத சமயத்தில் சாதாரண மாதுளம் பிஞ்சைக் கூடப் பிரயோகத்தில் சேர்த்துக்கொள்ளலாம்.

மாதுளம் பூ

"வாந்திபித்த தோடமொடு மாறாக் கடுப்பனலஞ்
சேர்ந்துநின்ற மூலரத்தந் தீர்க்குங்காண் - மாந்தளிர்க்கை
மாதே கறைப்புஷ்டி வல்லபல னுண்டாக்கும்
பூதலத்துன் மாதுளையின்பூ"

குணம்

மாதுளையின் பூ பித்தவாந்தி, வயிறளைதல், வெப்பம், ரத்தமூலம் இவைகளைப் போக்கும். ரத்தப் புஷ்டியையும் பலத்தையும் தரும்.

செய்கை

சங்கோசனகாரி, வியதாபேதகாரி

உபயோகிக்கும் முறை

மாதுளம் பூ, மாதுளம் பிஞ்சு, மாதுளம் தளிர் இவைகளைத் தனித்தனியாகவாவது (அ) ஒரு மிக்கச் சேர்த்தாவது அரைத்துத் தயிரில் கலக்கிக் கொடுக்க ரத்தபேதி, சீதபேதி முதலியவைகள் போகும்.

மாதுளை வித்து – சி. கண்ணுசாமி பிள்ளை, பதார்த்த குணவிளக்கம் மூலவர்க்கம் ப.எண். 593,594)

குணம்

இனிப்புள்ள மாதுளம் வித்தானது சுக்கிலத்தை இறுக்குவதுந் தவிர, அதிலுள்ள குற்றத்தையும் பிரமேகச் சுறுக்கையும் நீங்கும்.

செய்கை

சங்கோசனகாரி

உபயோகிக்கும் முறை

இதனைப் பசுவின் பால்விட்டு அரைத்துப் பாலில் கலக்கிச் சாப்பிட மேகக்கடுப்பு, சுக்கில நஷ்டம் முதலியவற்றைப் போக்குவதுடன் மலத்தைக் கட்டும்.

மாதளை சேரும் மருந்துகள்

1. சுண்டைவற்றல் சூரணம்

சுண்டைவற்றல், கறிவேப்பிலை, மாம்பருப்பு, ஓமம், நெல்லிவற்றல், மாதுளம்பழத்தோல், வெந்தம் - 1 பாகம்

செய்முறை

மேற்கூறிய சரக்குகளைக் காயவைத்து, பிறகு இளவறுப்பாய் வறுத்து நன்றாக இடித்துப் பொடித்து சலித்து வைக்கவும்.

அளவு

1-2 கிராம்

துணைமருந்து

எருமைத்தயிர்

தீரும் நோய்கள்

பொருமல், மந்தம், இரைச்சல், கழிச்சல், எருவாய் மூளைநோய், பெருங்கழிச்சல், நிணக்கழிச்சல்.

- தேரையர் கரிசல் 300 ப.எண்.88

2. மாதுளைநெய்:

- சிகிச்சாரத்தின் தீபம் (கண்ணுசாமி பிள்ளை)

அளவு :

1 காசு எடை – 2வேளை.

தீரும் நோய்கள்

சவலையினாலேனும், கணச்சூட்டினாலேனும், வேறு எக்காரணத்தினாலேனும் நாளுக்கு நாள் இளைத்து தோலும் எலும்புமாகக் காணப்படும் மக்களின் தேகம் கொழுமையைந்து 3 மாதத்திற்குள் எல்லோரையும் ஆச்சரியப்படும் படிச் செய்யும்.

பத்தியம்: இச்சாபத்தியம்

3.மாதுளை மணப்பாகு

"கற்கண்டும் பன்னீர் கனிமா துளைரசமும்
நற்றேனுங் கூட்டியே நாட்டுவாய் - சொற்பமல்ல
பாண்டு வெளுத்தலொடு பாதங் கரமெரிதல்
மாண்டுபோ மென்றார் மகிழ்"

கற்கண்டு, பன்னீர், மாதுளை இரசம், தேன் இவைகளை ஒன்றாகக் கூட்டிக் கற்கண்டு கரைந்த பின் வடிகட்டி, அடுப்பிலிட்டுக் கொதிக்க வைத்துப் பாகுபதம் வந்தவுடன் இறக்கி புட்டியில் அடைக்கவும். அளவுப்படி கொடுக்க பாண்டு, வெளுக்கும் நோய். கைகால் எரிவு, வாந்தி இவைகள் தீரும்.

4. ஏலாதிச் சூரணம் (சரபேந்திரர் சித்தமருத்துவச்சுடர் ப.எண்.557, 558)

அளவு	:	வெருகடியளவு (இருவேளை)
அனுபானம்	:	தாய்ப்பால், தேன், பழச்சாறு, மாதுளம் பழச்சாறு
தீரும்நோய்கள்	:	எரிவுடன் இரத்தம் வருதல், தாகவறட்சி, சோபம், வாந்தி, பித்தம், தலைவலி, அஸ்திச்சுரம், வாயு, ஈளை, இருமல், உப்பிசம், நெஞ்செரிவு முதலியன குணமாகும்.

5. ஐங்காயச் சூரணம் (சரபேந்திரர் சித்தமருத்துவச்சுடர் ப.எண்.559, 560)

அளவு	:	2 முதல் 5 கிராம் (2 வேளை)
அனுபானம்	:	தேன், சர்க்கரை
தீரும்நோய்கள்	:	அசீரணபேதி, கிராணி, வயிற்றுப்புசம், அக்கினிமந்தம், உஷ்ணபேதி முதலிய நோய்கள் குணமாகும்.
பத்தியம்	:	நோயின் வன்மைக்கேற்ப கஞ்சி, புனற்பாகம், பொரிகஞ்சி போன்றவைகளையே கொடுக்க வேண்டும். காரம், புளி சேர்க்கக்கூடாது.

6. கபாடச் சூரணம் ((சரபேந்திரர் சித்தமருத்துவச்சுடர் ப.எண்.560, 561)

அளவு : வெருகடியளவு (2 வேளை)

அனுபானம் : தேன், பனைவெல்லம்

தீரும் நோய்கள் : கிராணி, அதிசாரம், வயிற்றுளைச்சல், கழிச்சல், இரைச்சல், சிறுகுடல், பொருமல், தாகம், சோகம் முதலியன குணமாகும்.

7. மதன காமேசுரச் சூரணம் (சரபேந்திரர் சித்தமருத்துவச்சுடர் ப.எண். 581 - 584)

அளவு : வெருகடி அளவு (2 வேளை)
1 மண்டலம் (48 நாட்கள்)

தீரும் நோய்கள் : மேகவெட்டை, தாதுநட்டம், வாதசீதளம், குடைச்சல், முடக்கம், பித்தம், வாந்தி, மலபந்தம், வாயு, வாதம், குன்மம், மேகம், மூலக்கிராணி, பாண்டு, விஷபாகங்கள், குளிர்சுரங்கள் முதலியன குணம் அடையும். வீரிய விருத்தியாகும்.

8. கட்டுவாதிச்சூரணம் (கோஷாயி அனுபோக வைத்திய பிரம்ம ரகசியம் ப.எண்.106)

அளவு : 1 வராகனெடை

தீரும் நோய்கள் : அதிசாரம் - 6, சீதபேதி, கிரகணி 11 குணமாகும்.

MODERN ASPECT

EARTHWORM

MODERN ASPECT

Earth worms have been used in medicine for various remedies since1340A.D (Stephenson 1930). Describing earth worm Darwin notes "Earth worm is nature's wonderful creation. This unnoticed and unimportant small creature does such a useful and enormous job that we become dumbfound".

Dr. Berrett says, "The skeleton of earth worm's body has no structure but still it contains all the wonders of culture".

The use of earth worms as therapeutic drug is described in a book on ancient Chinese Medicine "shen Nong's Herbal". According to traditional Chinese medicine, earth worms possess anti pyretic, anti-spasmodic, diuretic and detox effect etc. In recent years it has also been found that earth worms have strong anti-asthmatic and anti-allergic effects (Mac et. a1 1964).

According to the description given by vohora and Khan (1978) earth worm have largely been used internally and externally as powerful aphrodisiacs. Internally there are useful in chronic cough, diphtheria and jaundice and for facilitating delivery. Earth worms increase body heat and are of value in rheumatism, bronchitis, nerve disorders and tuberculosis.

Poonagam is being used in siddha Medicine very succesfully for many years. An intensive literary collection was done through books and discussions made with eminent scholars of science. According to our Siddha and Modern literature, Poonagam mainly indicated in the disease of chronic cough, hemiplegia and rhematism.

Tamil Name	:	Poonagam
English Name	:	Earth Worm
Zoological name	:	lumbricus terrestris

SYSTEMIC POSITION

Phylum	:	Annelida
Class	:	Clitellata
Order	:	Lumbriculida
Family	:	Lumbricidae

Genus	:	lumbricus
Species	:	terrestris

DISTRIBUTION:

It is cosmopolitan in distribution. It is found abundantly in Europe, India, Srilanka, and north pacific.

HABITAT AND HABIT:

Lumbricus terrestris can inhabit all soil types except coarse sands, bare rock and acidic peat (Sphagnum). It has been found to be constrained by the -15°C isotherm. It tolerate soils with pH values as low as 3.5 – 3.7 and as high as about 8.L.terrestric is not frost-tolerant indicating that it hibernates in deep soil layers during the winner (Addison, 2009; Tiunov at al., 2006; Wironen & Moore, 2006). Although often present in agricultural fields, it fares poorly due to herbicides, mechanical damage and lack of leaf litter (L.Frelich, pers.comm.)

SHAPE AND SIZE:

The body is long, narrow, slender, bilaterally symmetrical, tapering posteriorly and relatively broad anteriorly, it is approximately dorsoventrally flattened with rounded dorsal surface and flat ventral surface.it may range from 30-40 cm in length and 2-6 mm in width.

COLOUR:

The colour of the worm is red or yellowish brown. The colour may also vary even in the individuals of same species of different age and sexual maternity.

SEGMENTATION:

The anterior end is differentiated into a distinct head and the rest of the body is divided by a series of segments arranged in a linear series.

EXTERNAL MORPHOLOGY:

The prostomium of lumbricus bears a pair of small tactile tentacles and a pair of stout palps. On the dorsal surface of the prostomium are two pairs of black eyes lying directly over the brain. Each eyes is a cup of modified epidermal cells, the ends of which extended through a black pigment layer to form a retinal lining of light

sensitive rods. The cavity is filled with a lens , protruding from the cup as a spherical swelling covered by a transport layer of skin, the cornea, the periosteum of lumbricus is actually two segments fused together. The body may be divided into as many as 200 segments.

BODY WALL:

It provides definite shape to the body (due to its elasticity). Moist body wall helps in respiration. Protects the internal delicate organ from injury. Secrete mucus which keeps body surface slimy and kills harmful bacteria. Alternative contraction and relaxation of circular and inner longitudinal muscle helps in movement. Albumen secreted by clitellar gland helps in nutrition of embryo developing inside cocoon. Sensory epidermal cells serve for reception of external stimuli.

INTERNAL MORPHOLOGY:

The mouth opens into a muscular pharynx which occupies several segments. The pharynx leads to a tubular esophagus into which a pair of glandular digestive pouches opens. The rest of the digestive system in nereis is a simple long intestine ending at a short rectum in front of the anus. The blood collected in to a longitudinal dorsal vessel and distributed from a longitudinal ventral vessels. At the anterior end several pairs of commissures around the pharynx and esophagus connect the two vessels. The large bilobed brain is in the prostomium of lumbricus. Many small nerves extend to all parts of the anterior end of the body.

HOW THE EARTH WORM SQUIRMS ALONG:

One set of muscles runs the length of the body. The other set runs around the body. The worm can make its body longer by relaxing the long muscles and shortening the other ones.

It can shorten its body by pulling in the long muscles and loosening the others. To more forward, it grips the dirt with the bristles under the tail end of its body. Then it lengthens the body. This action pushes the head forward through the dirt, then the worm grips the dirt under its head and pulls up the tail end of its body.

ANALYSIS OF FRESH EARTHWORMS:

Analysis of fresh earthworms gave the following values

Water	:	79.86%
Protein	:	10-14%
Fat	:	1.5% of the total fat
Glyceridic	:	56% - 67%
Phosphatidic	:	44 -33 %
Unsaponifiable matter	:	50%

Unknown acids in addition to fatty acids of the $C_{10} - C_{22}$ series

- Ergosterol
- Cholesterol
- Stigmasterol
- Flavin

The chief end product of the nitrogen metabolism was adenine. A yellowish amorphous substance which cause haemolysis in dilution of 1:8000 and a bronchodialating principle have also been isolated from earthworm.

A Vital Component of Traditional Chinese Medicine:

In China and other parts of Asia, earthworms are also used for their medicinal properties. Earthworms are caught, killed and then eviscerated, with the viscera and organic components washed away. The worms are then dried for use.

According to the concepts of traditional Chinese medicine, earthworm is associated with the Bladder, Liver, Lung and Spleen meridians, and has salty and cold properties. It drains liver heat and clears lung heat, and can also clear heat in the collateral channels. Typically, earthworm is used with other herbs to treat a wide range of conditions, ranging from spasms and convulsions to pain relief, treatment of fevers and certain types of arthritis. It is also used to treat some types of asthma and bronchitis.

The average recommended dose of earthworm is 4.5 to 12 grams per day as an oral decoction. Larger amounts (10-20 grams) are used when fresh earthworms are employed. As a powder, 1-2 grams are recommended for oral use.

Dried, powdered earthworm can be found at some Asian markets and specialty stores. Earthworm is also available as a decoction, usually as part of a larger herbal formula.

Because earthworm has a strong taste that can invariably be salty, “fishy” or both, it may cause nausea and even vomiting in some sensitive individuals. This can be countered by taking earthworm powder, or taking earthworm with citrus fruits or other herbs. In addition, some patients may experience allergic reactions to earthworm. It also should not be taken by Women who are pregnant or lactating. As of this writing, there are no known drug interactions with earthworm. As always, make sure to consult with a licensed health care provider before taking earthworms or any other herbal remedies or dietary supplements.

EARTHWORM TO REMOVE BLOOD CIRCULATION IMPEDIMENT :

A survey Chinese have used earthworms for thousand of years modern scientific research has found out why, it contain three thrombolytic enzymes: fibrinolysin (plasmin) profibrinolysing activator and collagenase which are all we need to dissolve thrombus and restore blood circulation to the brain.

Earthworms as Food in Different Cultures:

Earthworms have been used as food in many cultures. It is generally considered a delicacy and is reserved only for the most distinguished guests and the elderly. Now we know why.

NEW DISCOVERY AND A NEW MEDICINE:

During to 70s professor Shanhongren discovered enzymatic functions of extract from earthworms which confirms the validity of the use of earthworms in traditional Chinese medicine. In 1997 a product named plasmin made from earthworm was approved by the chinese government as a new medicine. In 1997 plasmin was endorsed by the china geronotology foundation and a year later it was endored by the china geronotology association rehabilitation committee. In 1999 china medical society decided to make plasmin a key product to be promoted all over China. In the same year it was registered by the china supervisory and administrative bureau as a class two nationally protected TCM formula, and in 2000 it is included in the china national pharmacopoeia.

TRADITIONAL USE OF EARTH WARMS:

Traditional Chinese medicine has been using earthworm (lumbrius) for thousands of years. The famous compendium materia medica describes, it as “ salty in

taste" cold in property, and efficacious in clearing the geart, invigorating blood circulation dissolving stasis, opening up channels, curing stroke, hemiplegia and infantile convulsion, and pointed out that lumbricus has self dissolving ingredients.

Modern scientific research, especially Prof.Shan's finding, verified the Chinese use of earthworms after finding important enzymes in them.

HOW IT WORKS:

Made of a special strain of earthworms with new biochemical, engineering technology, plasmin provides three thrombolytic enzymes; fibrinolysin (plasmin)., Profibrinolysin activator and collagenase, it is nontoic and good for long term use without any side effect. This enteric capsule carries plasmin into the intestines and opens up and is absorbed into blood circulation system. It helps to maintain a healthy balance between hemolysis and hemostasis, may help reduces the risk of ischemic cardiovascular problems,such as stroke, embolism, thrombus, arteriosclerosis, etc.,The risk of diabetic complications including nervous pathological changes and micro circulation disturbances, and maintain healthy arterial functions and promote blood circulation by opening up arteries and help to maintain a healthy cholesterol and blood sugar level, reduced blood fat. It also provides many trace elements and vitamins needed by the human body.

EARTHWORM BENEFITS:

- Improve the physical structure of the soil
- Improve soil fertility
- Improve plant growth and health
- A large earthworm population suppresses weed growth
- Worms often help clean up dangerous chemicals in the environment
- Improve water absorption and prevent erosion

POMEGRANATE

Botanical name : Punica granatum.Linn

According to Bentham & Hooker's classification Punica granatum is classified as follows:

Kingdom	:	Plant kingdom
Division	:	Angiosperms
Class	:	Dicotyledons
Sub class	:	Polypeptalae
Series	:	Calyciflorae
Order	:	Mystales
Family	:	Lythraceae
Genus	:	Punica
Species	:	Granatum

Vernacular names

Hindi	:	Anar
Beng	:	Palim
Mar	:	Palimba
Guj	:	Dadam
Tel	:	Danimma
Tam	:	Madulai
Kan	:	Dalimba
Mal	:	Matalam

Habitat

In moral tryst china, Afghanistan, Pakistan, Bangladesh, Iran Iraq, India, Burma, Spain, Moroko, Baluchistan, Arabia and California.

In India, Maharashtra, Gujarat, Rajasthan, Karnataka, Punjab, Haryana, Tamilnadu, Andra pradesh and Uttar Pradesh.

It is an attractive chrunb or small tree 6 to 10m tall, much branched, spiny and long lived. It has strong tendency to sucker from the base.

Leaves

Leaves are evergreen or deciduous, opposite or in words of 5-6 short stemmed oblong-calceolate and leathery.

Flower

Flowers are showy and characterized by the thick, tubular red calyx with pointed lepals. The flowers are bright red, 3cm in diameter, with four to five petals (often more on cultivated plants) . Some fruitless varieties are grown for the flowers alone. The edible fruit is a berry and is between a lemon and a grapefruit in size 5-12cm in diameter with a rounded hexagonal shape, and has thick reddish skin. The exact number of seeds in a pomegranate can vary from 200 to about 1400 seeds, contrary to some beliefs that all pomegranates have exactly the same number of seeds. Each seeds has a surrounding coater-laden pulp the edible aril-ranging in color from white to deep red or purple. The seeds are embedded in a white, spongy, astringent pulp.

Fruits

Fruits are round with a longly leathery skin, yellow, light or deep pink or dark red in colour. The inner portion is separated by membranous walls and white spongy tissue into compartments packed with transparent sacs filled with flavorful, fleshy, juicy, red, pink or whitish pulp. In each sac, there is one white or red, angular, soft or hard seed. The aril around the seeds from the ediple parts consist of delicious juice.

Actions:

Flower and rind

- Astringent
- Stomachic

Stem bark and root bark

- Anthelmintic

Fruit

- Refrigerant

- (குணப்பாடம் மூலிகை வகுப்பு ப.எண்.750)

Uses

- All parts of the tree can be utilized as source of tannin for curing leather.
- Both the wind and the flowers yield dyes for textile. The juice is rich in citric acid and sodium citrate and used in the treatment of dyspepsia and leprosy.

- The bark of the stem and root contains alkaloids like iso-pelletierine used against tape warms extracts of bark, leaves, imature fruits and fruit pind have been presecribed as astringent to half diarrhoea, dysentery & haemorrhages.
- Dried pulverized flower buds are administered against bronchitis. Detection of flowers is gargled to relieve oral and throat inflammation.
- Tracks of different parts of the tree exhibited antibiotic activity. Extracts of whole fruit highly active against micrococcus pyogenesvar aureus, E.coli and pseudomonas aeruginosa.
- Fresh pomegranate juice is used as an ingredient of cooling and refrigerant mixtures and of some medicines for dyspepsia.
- The rind is valued as an astringent in cases of diarrhoea & dysentery.
- The expressed juice of the leaves and the young fruit and the decoction of the bark are seed in dysentry.
- In java, an ink is sometimes arade from infusion of the leaves in native in egar.
- The sweet types of pomegranate said to be acidly laxative, white the less sweet types are believed to be good in inflammation of stomach and in head pain.
- The powered flower buds are used in bronchitis. The seeds are considered to be stomachic and the pulp cardiac and stomachic.

Handbook of Edible fruits Pg. No.402-404

In Ayurvedic medicine

- The rind of the fruit and the bark of the pomegranate tree is used as a traditional remedy against diarrhoea, dysentery and intestinal parasites.
- The seeds and juice are considered a tonic for the heart and throat and classified as a bitter-astringent (pitta or fire) component under the Ayurvedic system and considered a healthful counter balance to a diet high in sweet-fatty (kapha or earth) components.
- The astringents qualities of the flower juice, rind and tree bark are considered valuable for a variety of purposes, such as stopping nosebleeds and gum bleeds, toning skin, (after blending with mustard oil) firming up sagging breasts and treating hemorrhoids, pomegranate juice (of specific fruit strains) is also used as eyedrops as it is believed to slow the development of cataracts.

Pomegranate has been used as a contraceptive and abortifacient by means of consuming the seeds, or rind as well as by using the rind as a vaginal suppository.

This practice is recorded in ancient Indian literature, in Medieval sources and in modern folk medicine.

Potential health benefits

In preliminary laboratory research and clinical trials, juice of the pomegranate may be effective in reducing heart disease risk factors, including LDL oxidation, macrophage oxidative status and foam cell formation in mice, "oxidation of LDL by peritoneal macrophages was reduced by up to 90% after pomegranate juice consumption".

In a limited study of hypertensive patients, consumption of pemegranate juice for two weeks was shown to reduce systolic blood pressure by inhibiting serum angiotensin converting enzyme. Juice consumption may also inhibit viral infections while pomegranate extracts have antibacterial effects against dental plaque.

Despite limited research data, manufacturers and marketers of pomegranate juice have liberally used evolving research results for product promotion, especially for putative antioxidant health benefits. In February 2010, the FDA issued a warning letter to one such manufacturer, POM wonderful, for using published literature to make illegal claims of unproven antioxidant and anti – disease benefits.

Nutrients

That fiber, however, is entirely contained in the edible seeds which also supply unsaturated oils. People who choose to discard the seeds forfeit nutritional benefits conveyed by the seed fiber, oils and micronutrients.

Nutritive value (per 100gm of edible portion)

– The pomegranate Pg.No.1 Author-M.K.Sherth

Moisture	:	78gm
Protein	:	1.6gm
Fat	:	0.10gm
Minerals	:	5.10gm
Phosphorus	:	0.07gm
Iron	:	0.30gm
Riboflavin	:	100mg
Vitamin C	:	16mg

Seeds

- Astringent
- Anthelmintic
- Toenifuge
- Aphoridisiac

Climate & Soil

Pomegranate is basically a crop of acid and semiarid region and it requires hot dry summer and cold winter for production of quality fruits. A temperature of 35 to 38° is ideal for quality fruit production. Cool winter and hot summer is favourable for this crop. It can be grown in tropical and sub-tropical parts of North eastern hill region upto an attitude of 1000m from sea level. In a sub-tropical climate, it flowers in the spring but in tropical climate it flowers through out the year. However flowers of early rainy season give a quality in novermber-December.

- (Wealth of India Pg.No.317)

Propagation

Basal step cuttings with a diameter of 1.0 to 1.25 taken during rainy season and maximum success is achieved with the treatment of IBA at 5000ppm. It is also propagated by air layering. Air layering during rainy season and November-December gives profuse rooting.

Phytochemical study

- Pomegranate arial juice provides about 16% of an adults's daily vitamin C requirement pear 100ml serving, and is a good source of vitamin B5 (Pantothenic acid) potassium and natural phenols, such as ellagitannis and flavonoids.
- Analysis edible portion of pomegranates from coonoor give moisture-78.8, protein – 1.6, fat-0.4, fibre – 5.1, other carbohydrates 1405 & mineral matter 0.7% calcium 18, magnesium 12, oxalic acid 14, phosphorus 78.6, Iron – 0.3, sodium – 0.9, potassium – 33.6, copper – 0.2, sulphur-12. chlorine-2.8, carptene-8, thiamine – 0.06, ribothamine-0.10, elicobinicacid – 0.30 & vit C – 14 mg /100mg.

- The fresh rind of the fruit contains wax 0.8, resins – 4.5, mannitol – 1.8, non-crystallized sugars – 2.7, gums – 3.2, insulin – 1.8, mucilage – 0.6, mannin – 16.4, gallic acid – 4, calcium oxylate-4.8%
- The following alkaloids have been reported as occuring in the bark, Pelletierine ($C_8H_{15}ON$) – isopelletierin ($C_8H_{15}ON$) Pseudopelletierine ($C_9H_{15}ON$) Methyl Pelletreuine ($C_9H_{17}ON$) & Methyl isopelletierine ($C_9H_{17}ON$).
- D-Mannitol occurs abundantly in the stem bark and in lesser amounts in rootbark seeds & leaves.
- Biological tests indicate that mannitol possess mild anti spasmodic & anthelminetic properties.
- Frieldelin & betulic acid are reported in the bark, betulic and ursolic acid in the leaves and ursolic acid in the fruit kind.

LATERAL RESEARCH WORKS

PHYTO-CHEMICAL STUDIES

Two new b-sitosterol esters have been isolated from the flowers of punica granatum Linn. (punicaceae) along with the known compounds n-tricosane(3), nheptacosanyln-hexanoate (4), olean-5, 12-dien-3b-ol-28-oic acid (5), and olean-12-en-3b-ol-oicacid (6). The structures of the new phyto sterols have been elucidated as stigmast – 5-en-3b-ol-3b-dodecanoate(6-sitosterollaurate, 1) and stigmast-5-en-3b-ol-3b-tetradeconoate.

PHARMACOLOGICAL ACTIVITIES

ANALGESIC ACTIVITY

The extracts of flowers of Punicagranatum (Linn). (N.O.Family Punicaceae) were investigated for analgesic activity in mice using hot plate method. The flowers of Punicagranatum (Linn) were collected from the local market of Mumbai, Maharashtra and were in a dried condition. The dried powdered flowers (500gm) were extracted in a soxhlet apparatus by using different solvents mice weighing 15-25gm were taken for the experiment. The reaction time of animals in all the groups was noted at 30, 60 and 120 min after drug administration. All data were analyzed with student-t-test. The various extract of the flowers of punicagranatum (Linn). showed significant analgesic activity at a dose of 50mg/kg body weight. A maximum analgesic activity was found at 60min, after drug administration, which was equivalent to the standared drug used as morphine sulphate.

WOUND HEALING ACTIVITY

The present study demonstrated that Punicagranatum extract was capable of promoting. Enhanced wound contraction and histological observations suggest that punicagranatum has potential in the management of wound healing and suggests further study.

ANTI-SPASMODIC ACTIVITY

Our result indicated that aqueousand hydroalcoholic extracts of punicagranatum flower could induce relaxant effects on uterus of virgin rat and uterine contractions were decrease without involvement of β-adrenoceptors or opioidreceptors. These results indicate that aqueous extracts of punicagranatum flower could induces spasmolytic effect on rat uterus through blockage of VDCCs.

These results support the clinical efficacy and use of punicagranatum flower in the treatment of dysmenorrhoea and other uterine spasmodic disorders. This process appears to be the most relevant physiological process and should be the target of future research.

ANTI-DIABETIC ACTIVITY

Peroxisome prolifecator-activated receptor (PPAR)-gamma activators are widely used in the treatment of type & diabetes because they improve the sensitivity of insulin receptors. Punicagranatum flower (PGF) has been used as an anti-diabetic medicine in unani medicinal literature. The mechanism extract diminishes cardiac fibrosis in zucker diabetic fatty rats, atleast in part, by modulating cardiac ET-1 and NF-kappa B signaling of actions is, however, unknown. In the current study, we demonstrated that 6-week oral administration of methanol extract from PGF (500mg/kg, daily) inhibited glucose loading-induced increase of plasma glucose levels in zucker diabetic fatty rats (ZDF), a genetic animal model for type 2 diabetes, whereas it did not inhibit the increase in zucker lean rat (ZL). The treatment didnot lower the plasma glucose levels in fasted ZDF and ZL rats. Further more, RT-PCR results demonstrated that the PGF extract treatment in ZDF rats enhanced cardiac PPAR-gamma MRNA expression and restored the down-regulated cardiac glucose transporter (GLUT)-4 (the insulin-dependent isoform of GLUTs) MRNA. These results suggest that the anti diabetic activity of PGF extract may result from improved sensitivity of the insulin receptor. From the in vitrostudies, we demonstrated that the PGF extract enhanced PPAR-gamma MRNA and protein expression and increased PPAR-gamma dependent MRNA expression and activity of lipoprotein lipase in human THP-1-differentiated macrophage cells. Phytochemical investigation demonstrated that gallic acid in PGF extract is mostly responsible for this activity. Thus, our findings indicate that PPAR-gamma is a molecular target for PGF extract and its prominent component gallic acid, and provide a better understanding of the potential mechanism of the anti-diabetic action of PGF.

CARDIAC ACTIVITY

The diabetic heart shows increased fibrosis, which impairs cardiac function. endotholin (ET)-1 and nuclear factor-kappa B (NF-kappa B) interactively regulate fibroblast growth. We have recently demonstrated that punicagranatum flower (PGF), a unani anti-diabetic medicine, is a dual activator of peroxisome proliferator-activated receptor (PPAR)-alpha and –gamma, and improves hyperglycemia, hyperlipidemia, and fatty heart in zucker diabetic fatty (ZDF) rat, a genetic animal model of type 2 diabetes and obesity. Here, we demonstrated that six-week treatment with PGF extract (500mg/kg, P.o) in zucker diabetic fatty rats reduced the ratios of van Gieson-stained interstitial collagen deposit area to total left ventricular area and perivascular collagen deposit areas to coronary artery media area in the heart. This was accompanied by suppression of overexpressed cardiac fibronectin and collagen I and III MRNAS. Punicagranatum flower extract reduced the up regulated cardiac MRNA expression of ET-1, ETA, inhibitor-kappa B beta and C-jun and normalized the down-regulated MRNA expression of inhibitor-kappa Balpha in zucker diabetic fatty rats. In vitro, Punica granatum flower extract and its components oleanolic acid, cusolic acid and gallic acid inhibited lipopolysaccharide-induced NF-kappa B activation in macrophages. Our findings indicate that punica granatum flower.

HEPATO-PROTECTIVE ACTIVITY

PGF- treated ZDF rats showed reduced ratio of liver weight to tibia length, hepatic triglyceride contents and lipid droplets. These effects were accompanied by enhanced hepatic gene expression of Peroxisome proliferator -activiated receptor (PPAR) – alpha, carnitine palmitoyl transferase- 1 and acyl-COA oxidase (ACO) and reduced stearoyl – COA dsaturase – 1. In contrast, PGF showed minimal effects on expression of genes responsible for synthesis, hydrolysis or uptake of fatty acid and triglycerides. PGF treatment also increased PPAR-alpha and ACO MRNA levels in HepG2 cells.

HONEY

Honey is a naturally converted form of sugary food, the nectar of flowers and other plant exudations systemically collected and stored by honey bees.

Honey as a vehicle:

Physical properties of honey

Aroma	:	Depends upon the floral source from where it's collected.
Colour	:	It ranges from pale yellow or yellowish brown to dark brown.
Specific rotation	:	+3 to -10

Constituents of honey

Honey contains chiefly dextrose and fructose moisture, small amounts of sucrose and mineral constituents.

- Moisture: 20.6% fructose: 38% glucose : 31% sucrose: 1% other sugar 8.5% fat: 0.1% minerals: 0.2% , Protein 0.3% others: 0.4%.
- Vitamins present in honey are vitamin B1-B6, Vitamin C.
- Minerals include calcium, phosphorous and Iron.
- Trace elements in honey arte magnesium, selenium, sulphur, chloride and silica.
- Coloring matter: Caroline, chlorophyll, xanthophylls, anthocyanin, tanin.
- Acids: Acetic acid, formic acid, malic acid, citric acid, succinic acid, oxalic acid.
- Enzymes: Chief enzyme present in honey is invertase. Other include protease, oxidases, peroxidase, reductase.

-The wealth of India Vol. II Pg.91)

Action

- Antimicrobial
- Antiseptic
- Sedative
- Demulcent

Uses of Honey

Provides wholesome nourishment also an energy giving rich food.

The predigested sugars present in honey are easy to digest and are readily absorbed and assimilated by the body.

Extensively used in preparing breads, cakes, biscuits, pastries, chewing gums, candies.

Honey constitutes all important ingredients of certain lotions, cosmetics, soaps, creams, balms, inhalations.

Distinguishing quality honey

High quality natural honey can be distinguished its fragrance and taste. The honey should not lay down in layers. It this is a case, it indicates the excessive humidity (over 20%) of the product, and such a honey would not be suitable for long term preservation.

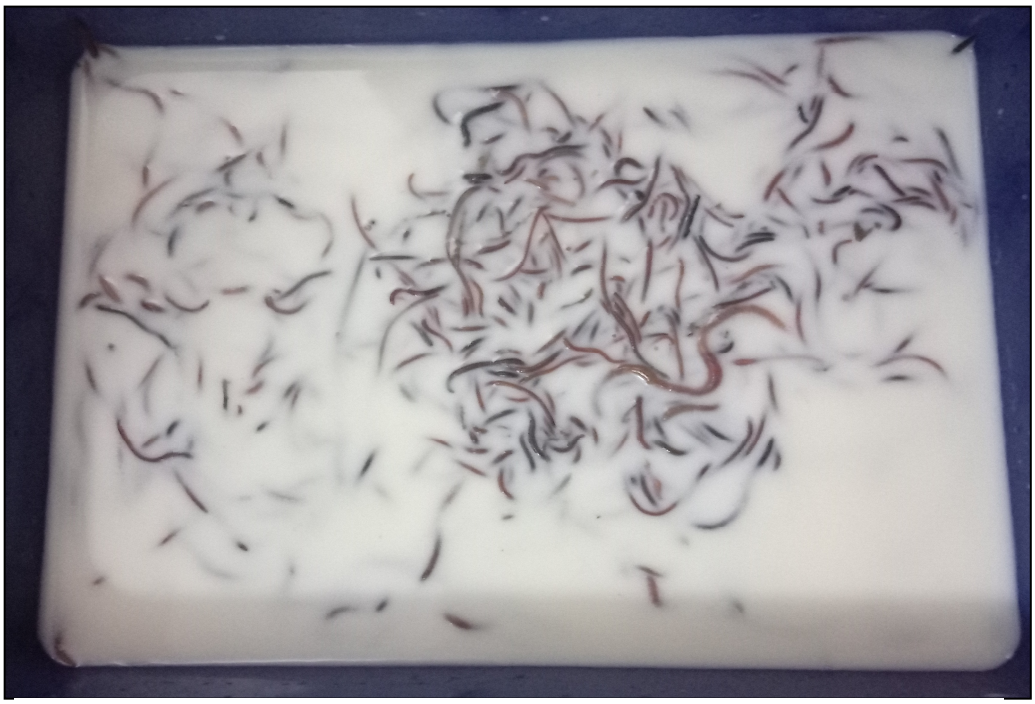
A fluffy thin layer on the surface of the honey (like a white foam) or marble-coloured and white spots in crystallized honey at the wall sides of the bottle are caused by filling of liquid honey with subsequent sealing- the air bubbles are surfacing and part of them is concentrated at the wall sides. This is an indication of a high quality honey, which was tilled without pasteurization (heating).

A true honey that is at least one month old is usually of demure (not translucent) colour.

Preservation of Honey:

Because of its unique composition and the complex processing of nectar by the bees.

INGREDIENTS OF DRUG POONAGA PARPAM



Purification process of Poonagam

Purified Poonagam

INGREDIENTS OF DRUG POONAGA PARPAM

PULIPPU MATHULAI

KAVASAM

DRUG PREPARATION

PREPARED DRUG POONAGA PARPAM

4. MATERIALS AND METHODS

Selection of drug:

Poonaga Parpam mentioned in Anuboga vaidhya navaneetham (Part – 3, Pg.No. 117,118, Hakim P. Mohamed Abdulla Sahib) was selected for evaluating the toxic effect and mortality when given in short and long duration.

Collection of raw drug:

The raw drugs were collected from Agricultural land at Sankarankovil through proper identification.

Ingredients of poonaga parpam:

- Poonagam
- Sour pomegranate juice

Purification of raw drugs:

POONAGAM:

Poonagam soaked in milk for a day. Mud comes out from it.

Then wash it in water.

Method of preparation:

The purified drugs were taken in the following ratio.

- Purified Poonagam - 2 palam (70 gm)
- Sour pomegranate juice - S.Q

Purified poonagam was powdered and add sour pomegranate juice little by little. Grind for 2 Saamam and make cake pieces then dry it. Then it is placed into earthen plate and covered with another earthen plate, make 3 seelai and subjected into sublimation process by using cow dung, weight of twenty fold of kavasam.Then cool and obtain reddish shadow parpam and stored it in air tight container.

Dosage:

2 – 4 Kunrimani edai (260-520 mg)

Adjuvant:

Honey

5. QUALITATIVE AND QUANTITATIVE ANALYSIS

PHYSICOCHEMICAL ANALYSIS

Sample Description : ***POONAGA PARPAM***

Equipment used : Atomic Absorption Spectrometer (AAS)

Colour:

About 50gm of ***POONAGA PARPAM*** was taken in a clean glass beaker and tested for its colour by viewing again a water opaque background under direct sunlight.

pH:

The pH of ***POONAGA PARPAM*** was estimated as per the method prescribed in Indian Standard (IS) – 6940 (1982). One gram of the ***POONAGA PARPAM*** was taken into a 100ml graduated cylinder containing about 50ml of water and filled up to the mark with water. The cylinder was stopped and shaken vigorously for two minutes and the suspension was allowed to settle for an hour at 25^0 to $27^{0.}$ About 25ml of the clear aqueous solution was transferred into a 50ml breaker and tested for pH using DIGISUN digital pH meter (DIGISUN Electronics, Hyderabad, India)

Determination of Ash Value:

Weighed accurately 2 grams of ***POONAGA PARPAM*** in tarred platinum or silica dish and incinerate at a temperature not be exceeding 450^0C until free from carbon, cooled and weighed. Calculate the percentage of ash with reference to the air dried drug.

Water Soluble Ash:

To the gooch crucible containing to the total ash, added 25ml of water and boiled for 5 minutes. Collected the insoluble matter in a sintered glass crucible or on ash less filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature nor exceeding 450^0 C subtract the weight of the insoluble matter from the weight of the ash the difference of the weight represents the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug.

Acid Insoluble Ash:

Boiled the ash 5 minutes with 25ml of 1:1 dil HCL. Collect the insoluble matter in gooch crucible on an ash less filter paper wash with hot water and ignite. Cooled in a desiccators and weighted calculated the percentage of acid insoluble ash with reference to the air dried drug.

Loss on Drying:

Five grams of ***POONAGA PARPAM*** is heated in a hot oven at 105^0C to constant weight and the percentage of loss of weight has calculated there from.

PHYTOCHEMICAL ANALYSIS

PROCEDURE

Test for Alkaloids (Ansari, 2006)

The extract was evaporated in a test tube. To the residue dilute HCL was added, shaken well and filtered.

1.Mayer's Test:

To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitation showed the presence of alkaloids.

Ansari, S. H. 2006.

Essentials of pharnacognosy, 1st edition, Birla publications, New Delhi. pp. 357-359, 588-590.

2.Dragendroff's Test:

To 2 mg of the ethanolic extract 5 ml of distilled water was added, 2M Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicates the presence of alkaloids.

3.Hager's Test:

To 2 mg of the ethanolic extract taken in a test tube, a few drops of Hager's reagent was added.Formation of yellow precipitation confirms the presence of alkaloids.

Test for Carbohydrates and Glycosides

1.Molisch Test

2 mg of ethanolic extract was shaken with 10ml of water, filtered and the filtrate was concentrated. To this 2 drops of freshly prepared 20% alcoholic solution of α - naphthol was added. 2 ml of conc. sulphuric acid was added so as to form a layer below the mixture. Redviolet ring appear, indicating the presence of carbohydrates which disappear on the addition ofexcess of alkali.

2.Legal's Test

The test is employed for digitoxose containing glycosides. The extract of drug is dissolved in pyridine, sodium nitroprusside solution is added to it and made alkaline, pink or red color is produced.

3.Borntrager's Test

Borntrager's test is employed for presences of anthraquinones. The drug is boiled with dilute sulphuric acid, filtered and to the filtrate benzene, or ether or chloroform is added and shaken well. The organic layer is separated to which ammonia is added slowly. The ammoniacal layer shows pink to red color due to presences of anthraquinone glycosides.

Test for PhytoSteroids (IP, 1996)

1.Salkowski Test:

To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Indian Pharmacopoeia (IP). 1996.

Govt. of India, Ministry of Health and Family Welfare Published by the Controller of Publications, New Delhi, A-47, A-53, A-54.

2.Liebermann-Burchard's test

2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicates the presence of steroids.

Test for Flavanoids (Kokate, 1994)

Shinoda Test:

To the extract, 5 ml of 95% ethanol and few drops of concentrated hydrochloric acid was added. To this solution 0.5 gm of magnesium turnings were added. Pink colouration indicated the presence of flavanoids.

Kokate, C. K. 1994.

Practical Pharmacognosy, 4th edition, Vallabh Prakashan, New Delhi. 4 - 29.

Test for Tannins (Mukherjee, 2002)

Lead Acetate Test:

On addition of lead acetate solution to the extract white precipitate appeared.

Mukherjee, P. K. 2002.

Quality control of herbal drugs, business horizons pharmaceutical publishers, New Delhi. 356 - 358.

Saponin (Ansari, 2006)

Foam Test:

Drug extract was shaken vigorously with water. No persistent foam was formed.

QUANTITATIVE ANALYSIS OF POONAGA PARPAM

PROCEDURE:

Quantitative Estimation of carbohydrate

The total sugar content was estimated by Anthrone method (Roe, 1955). A known amount of the sample was taken, ground well with 80% ethanol and was centrifuged at 4000 rpm. From the supernatant, 0.5 ml was taken and 5 ml of anthrone reagent was added. The tubes were kept in a boiling water bath for 15 min. After that, they were kept in a dark room for another 15 minutes. The colour intensity developed was read in a spectrophotometer at 650 nm.

Ref: ROE, J. H. (1955), "The determination of sugar in blood and spinal fluid with anthrone reagent" Ibid., ill: 335-343.

Quantitative Estimation of flavanoids: (Evans, 1996)

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Ref: Devanaboyina N et al., "Preliminary Phytochemical Screening, Quantitative Estimation And Evaluation Of Antimicrobial Activity Of Alstoniamacrophylla Stem Bark" ***IJSIT, 2013, 2(1), 31-39***

Quantitative Estimation of Saponins: (Evans, 1996)

Methanolic and water extract was dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 600c for 10min, absorbance was measured at 544nm against reagent blank. Diosgeninis used as a standard material and compared the assay with Diosgenin equivalents.

Ref: Devanaboyina N et al., "Preliminary Phytochemical Screening, Quantitative Estimation And Evaluation Of Antimicrobial Activity Of Alstoniamacrophylla Stem Bark" ***IJSIT, 2013, 2(1), 31-39***

Quantitative Estimation of Tannins: (Robert, E.B. 1971. Agro.J.63, p.511)

1ml of the extract was mixed with 5ml of vanillin hydrochloride reagent (mix equal volumes of 8% HCL in methanol and 4% vanillin in methanol). The mixed was allowed to stand for 20mins and measure the absorbance at 500nm. The standard graph was plotted for working standard catechin solution (0 to 250µg/µl).

Ref: *Robert*, *EB*, "Method for estimation of tannin in grain sorghum ", *Agro J* , vol. *63*, *1971*,*p.511*; 10.

FOURIER TRANSFORM – INFRA RED SPECTROSCOPY

PERKIN ELMER – SPECFTRUM ONE

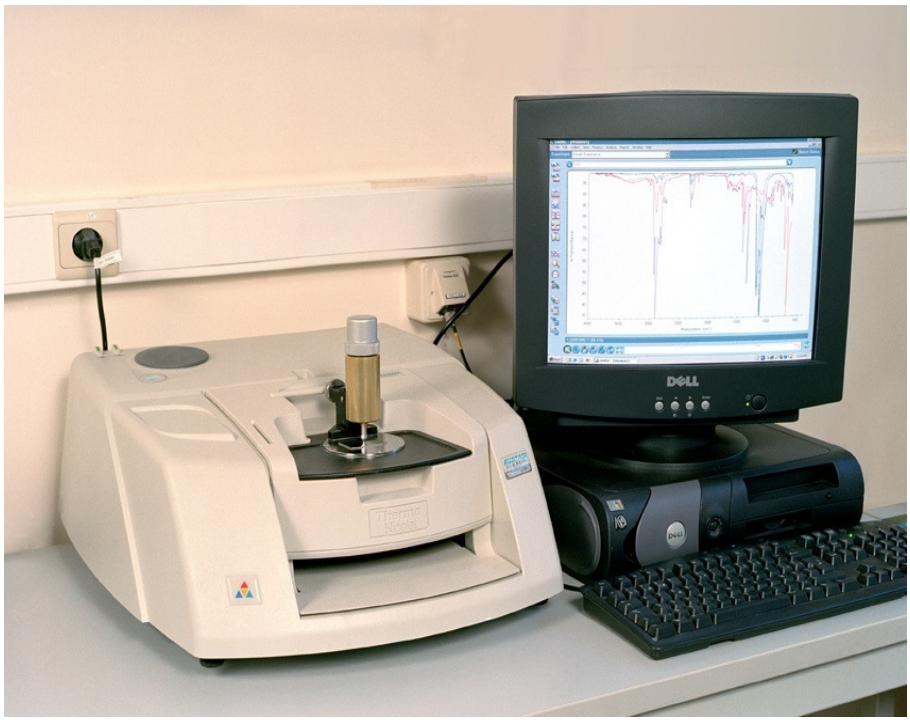
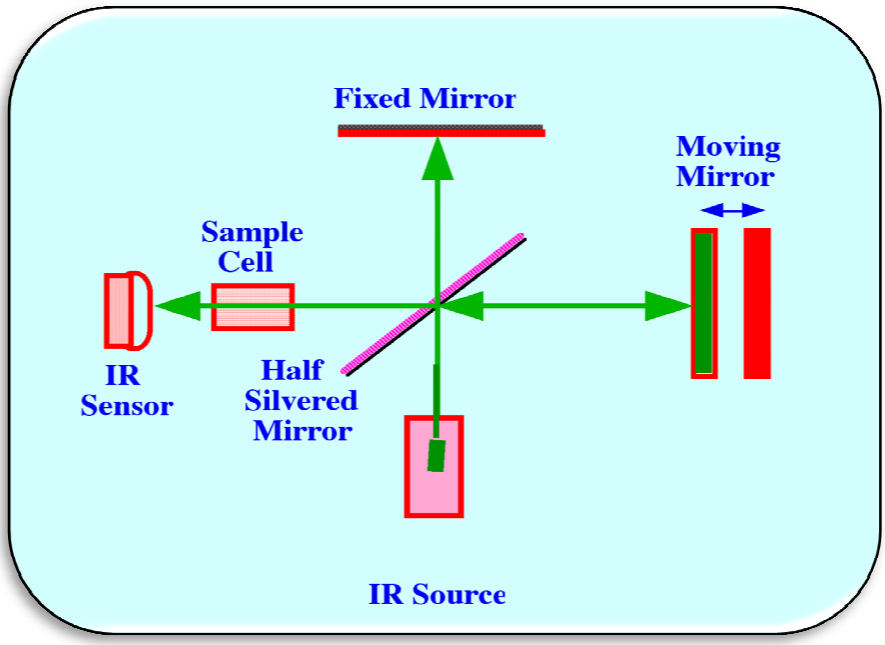


Fig. 3: FTIR Apparatus

FTIR-Mechanism



FOURIER TRANSFORM – INFRA RED SPECTROSCOPY
PERKIN ELMER – SPECFTRUM ONE

Introduction

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure. The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy.

In IR spectroscopy, the resonance absorption in made possible by the change in dipole moment accompanying the vibrational transition. The infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

Principle

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction,l electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecurlar and electronic structure of the substance and with intra – and inter molecular interactions.

FT-IR spectroscopy is used primarily for qualitative and quantitative analysis of organic compounds, and also for determining the chemical structure of inorganic materials. The region between 500-4000 wave number is referred to as the finger print region. Absorption bands in this region are generally due to intra molecular phenomena and are highly specific for each material. The specificity if these bands allow computerized data searches to be performed against reference libraries to identify a material.

Table of Characteristic IR Absorptions

Frequency, cm-1	**Bond**	**Functional group**
3640 - 3610 (s, sh)	O-H stretch	Free hydroxyl alcohols phenols
3500 - 3200 (s,b)	O-H stretch, H – bonded	Alcohols, phenols
3400 – 3250 (m)	N – H stretch	Primary, secondary, amines, amides
3300 – 2500 (m)	O – H stretch	Carboxylic acids
3330 - 3270 (n, s)	–C (triple bond) C – H : C - H stretch	Alkynes (terminal)
3100 – 3000 (s)	C – H stretch	Aromatics
3100 – 3000 (m)	= C – H stretch	Alkenes
3000 – 2850 (m)	C – H stretch	Alkenes
2830 – 2695 (m)	H – C = 0; C –H stretch	Aldehydes
2260 - 2210 (v)	C (triple bond) N stretch	Nitriles
2260 – 2100 (w)	C (triple bond) C- stretch	Alkynes
1760 – 1665 (s)	C = 0 stretch	Carbonyls (general)
1760 – 1690 (s)	C = 0 stretch	Carboxylic acids
1750- 1735 (s)	C = 0 stretch	Esters, saturated aliphatic
1740 – 1720 (s)	C = 0 stretch	Aldehydes, saturated aliphatic
1730 – 1715 (a)	C = 0 stretch	Alpha, beta – unsaturated esters
1715 (s)	C = 0 stretch	Ketones, saturated aliphatic
1710 – 1665 (s)	C = 0 stretch	Alpha, beta – unsaturat aldehydes, ketones
1680 – 1640 (m)	-C = C -	Alkenes
1650 – 1580 (m)	N – H bend	Primary amines
1600 – 1585 (m)	C-C stretch (in – ring)	Aromatics
1550 – 1475 (s)	N – 0 asymmetric stretch	Nitro compounds
1500 – 1400 (m)	C –C stretch (in – ring)	Aromatics
1470 – 1450 (m)	N – 0 asymmetric stretch	Nitro compounds
1370 – 1350 (m)	C – H bend	Alkanes
1360 – 1290 (m)	C – H rock	Alkanes

1335 – 1250 (s)	C – N stretch	Aromatic amines
1320 – 1000 (s)	C – 0 stretch	Alcohols, carboxylic acids, esters, ethers
1300 – 1150 (m)	C – H wag (- CH2X)	Alkyl halides
1250 – 1020 (m)	C – N stretch	Aliphatic amines
1000 – 650 (s)	=C – H bend	Alkynes
950 – 910 (m)	O – H bend	Carboxylic acids
910 – 665 (s, b)	N – H wag	Primary, secondary amines
900 – 675 (s)	C – H "oop"	Aromatics
850 – 550 (m)	C – CI stretch	Alkyl halides
725 – 720 (m)	- C (triple bond) C-H : C- H bend	Alkynes
690 – 515 (m)	C - Br stretch	Alkyl halides

M = medium, w = weak, s=strong, n = narrow, b = broad, sh = sharp

Sampling techniques:

There are a variety of techniques for sample preparation depending on the physical form of the sample to be analyzed.

Solid : KBr or Nujol mull method

Liquid : CsI / TIBr Cells

Gas : Gas Cells

Experimental Procedure: Done at SAIF, IIT Madras, Chennai – 36KBr Method

- The Sample was grounded using – an agate mortar and pestle to give a very fine powder.
- The finely powder sample was mixed with about 100 mg dried KBr salt.
- The mixture was then pressed under hydraulic press using a dye to yield a transparent disc (measure about 13 mm diameter and 0.3mm in thickness), through which the beam of spectrometer passed.

HR SEM - METHODOLOGY

HR SEM-Methodology:

An SEM is essentially a high magnification microscope, which used a focused scanned collection beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross-sections. The primary electron beam interacts with the sample in a number of key ways:-

Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen.

Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast.

Ionized atoms can relax by electron shell-to-shell transitions. Which lead to either X-ray emission or Auger electron ejection. The X-rays emitted are characteristic of the elements in the top few urn of the sample.

Sample Preparation:

Sample preparation can be minimal or elaborate for SEM analysis depending on the nature of the samples and the data required. Minimal preparation includes acquisition of a ***POONAGA PARPAM*** that will fit into the SEM chamber. And it should be analyzed.

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES),

Fig. 5: ICPOES Apparatus

ICP OES METHODOLOGY:

ICP, abbreviation for Inductively Coupled Plasma, is one method of optical emission spectrometry, When plasma energy is given to an analysis sample from outside, the component elements (atoms) is excited. When the excited atoms return to low energy position, emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays, and the content of each element is determined based on the ray's intensity.

To generate plasma, first, argon gas is supplied to torch coil, and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas in ionized and plasma is generated. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation –emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube.

Sample preparation:

Solids cannot be analyzed directly. Such samples should be made into clear aqueous medium quantitatively. When acids are used to prepare solutions care should be taken. The concentration of the acids in the final provided solution should not be

more than 2% v/v. highly acidic and organic solutions cannot be analyzed. As a guide line weigh exactly, around 200mg of substance and dissolve in 5mL of 5% of water or aquaregia or whatever acid to make 100mL of final solution. Make proper dilutions, if necessary. Free HF should not present in the final solution to be aspirated.

Ideal concentration is around 100 ppm of the element of interest. Total dissolved solids should be not more than 0.2% w/v in the final solution Very dilute solution may not give reliable results. Each element has a detection limit. A minimum solution volume of 25 ml is necessary for analysis.

In ICP intensity of light emitted when the sample "sprayed or aspirated into an argon plasma" is measured at different wavelengths. The intensity of light at a given wavelength will be proportional to a particular elemental ion concentration. The intensity is calibrated with known standard concentration. For accurate quantitative results It is necessary to simulate the sample matrix condition with that of the standard. Each element generally will have many emission lines and the sensitivity is different for each of this wave length. When more than one element is present it is quite common that some emission lines interfere due to overlapping.

It is preferable to use plastic containers for sample handling and preserving samples for **ICP-OES** analysis. Glass containers can give problems especially when analyzing certain metal ions at low concentration.

The samples of ***POONAGA PARPAM*** was prepared.

BIOCHEMICAL ANALYSIS

BIOCHEMICAL ANALYSIS OF POONAGA PARPAM:

Preparation of extract:

100mg of parpam is weighted accurately and placed into a clean beaker added a few drops of conc.hydrochloric acid and evaporated it well. After evaporation cooled the content and added a few drops off concentrated nitric acid and evaporated it well. After cooling the content add 20ml of distilled water and dissolved it well. Then it is transformed it to 100ml volumetric flask and made upto 100ml with distilled water. Mix well. Filtered it. Then it is taken for analysis.

Qualitative analysis:

S.No.	EXPERIMENTS	OBSERVATION	INFERENCE
1.	**Test for calcium:** 2 ml 0f the above prepared extract taken in a clean test tube to this add 2 ml of 4% ammonium oxalate solution.	Formation of white colour precipitate	presence of calcium
2.	**Test for sulphate:** 2 ml of the extract is added to 5% barrium chloride solution.	Formation of white colour precipitate	Presence of sulphate.
3.	**Test for chloride:** The extract is treated with silver nitrate solution.	Formation of white colour precipitate	Presence of chloride.
4.	**Test for carbonate:** The substance is treated with concentrated HCL.	Formation of effervescence.	presence of carbonate.
5.	**Test for starch:** The extract is added with weak iodine solution.	Formation of blue colour	presence of starch.
6.	**Test for ferric iron:** The extract is acidified with glacial acetic acid and potassium ferro cyanide.	Formation of blue colour	presence of ferric iron.

7.	**Test for ferrous iron:** The extract is treated with concentrated nitric acid ammonium thiocyanide solution.	Appearance of blood red colour.	presence of ferrous iron
8.	**Test for phosphate:** The extract is treated with ammonium molybdate and concentrated nitric acid.	Formation of yellow precipitate	presence of phosphate.
9.	**Test for albumin:** The extract is treated with esbach's reagent.	Formation of yellow precipitate	presence of albumin.
10.	**Test for tannic acid:** The extract is treated with ferric chloride.	Formation of blue black precipitate.	presence of tannic acid.
11.	**Test for unsaturation:** Potassium permanganate solution is added to the extract.	It gets decolourised.	Presence of unsaturated compounds.
12.	**Test for the reducing sugar:** 5ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minutes.	Colour change occurs.	Presence of reducing sugar.
13.	**Test for amino acid:** One or two drops of the extract is placed on a filter paper and dried well. After drying, 1 % ninhydrin is sprayed over the same and dried it well.	Appearance of Violet colour	Presence of amino acid.
14.	**Test for zinc:** The extract is treated with potassium ferro cyanide.	Formation of white precipitate	Presence of zinc.

6. PRE-CLINICAL STUDIES

TOXICITY STUDY

TOXICOLOGICAL STUDIES ON POONAGA PARPAM

OBJECTIVES

The aim of this study is to evaluate the toxicity of the drug POONAGA PARPAM, when administered orally to male Wistar Albino Rats with different doses, so as to provide a rational base for the evaluation of the toxicological risk to man and indicate potential target organs.

Guidelines followed:

OECD Guidelines No.423.

The experimental protocol was approved by IAEC (Institutional Animal Ethical Committee) as per the guidance of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forest, Government of India.

Study design and Controls:

1) Wistar Albino Rats in controlled age and body weight were selected.
2) POONAGA PARPAM was administered at 5mg/kg, 50mg/kg, 300mg/kg, 2000 mg/kg body weight as water as suspension along with blank.
3) The results were recorded on the day of drug administration approximately 1^{st} , 3^{rd} , 4^{th}, 24^{th} hours in post dosing further made in to observation upto 14 days.
4) The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at 22 ±3ºC and the relative humidity was maintained between 30-70% with 100% exhaust facility.

EXPERIMENTAL PROCEDURE

Animals

Male Wistar albino rats (150 – 200 gm) were used for the study. The animals were obtained from animal house, Kerala Veterinary and Animal Sciences, Mannuthy, Kerala. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light:dark cycle

was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee, Nandha College of Pharmacy, Erode (688/PO/Re/S/02/CPCSEA) and were in accordance with the Institutional ethical guidelines (Proposal Number:NCP/IAEC/2018-19/23)

Test Compound

Poonaga parpam

Administration Procedure

Honey was used as vehicle and various doses of Poonaga parpam were administered through oral route using gastric gavage tubes to animals by suspending in vehicle.

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table-1 Group Numbering and Identification animals were marking on body

Group No	Animal Marking
1	Head
2	Body
3	Tail

The group no., sex of the animal and animal numbers were identified as indicated below using cage label and body marking on the animals.

Table – 2 Numbering and Identification cage label and body marking on the animals.

Cage No	Group No	Animal marking	Sex
1	I	H,B,T	Female
2	II	H,B,T	Female
3	III	H,B,T	Female
4	IV	H,B,T	Female
5	V	H,B,T	Female

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

ACUTE TOXICITY STUDY

Acute Toxicity Studies

Acute toxicity studies were performed according to OECD-423 (Organization of Economic and Cooperation Development) guidelines.

Male Wister albino rats were selected by random sampling technique were employed in this study. The animals were fasted for 4 hr with free access to water. The Poonaga parpam was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 1000 and 2000 mg/kg) doses of the Poonaga parpam were employed for further toxicity studies. The following general behaviour was also observed during the acute toxicity study (Ecobichon DJ, 1997).

3.1. Doses:

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighted and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

Table – 3. Doses

GROUP	DOSE
Group –I	Control
Group – II	5mg/kg
Group – III	50mg/kg
Group – IV	300mg/kg
Group – V	2000mg/kg

The test substance was administered as single dose. After single dose administration period, all animals were observed for 14 days.

General Behaviours

S.No	General Behaviour
1	Sedation
2	Hypnosis
3	Convulsion
4	Ptosis
5	Analgesia
6	Stupar Reaction
7	Motor activity
8	Muscle Relaxant
9	CNS Stimulant
10	CNS Depressant
11	Pilo Erection
12	Skin Colour
13	Lacrimation
14	Stool Consistancy

SUB-ACUTE TOXICITY STUDY

1.Objective

The objective of this 'Sub-acute toxicity study of POONAGA PARPAM on Wistar Albino Rats' was to assess the toxicological profile of the test substance when treated as a single dose. Animals should be observed for 28 days of drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

2. Test Guideline followed

OECD 407 Method – Sub-Acute Toxic Class Method (Repeated Dose 28-Day Oral Toxicity Study in Rodents)

3. Test substance Detail

Name: POONAGA PARPAM

Wistar albino rats of either sex weighing 150-200g were used in the study. The animals were divided in to 3 groups of 6 animals each. Group I served as control received Distilled water (1ml/kg). Group II & III received the Poonaga parpam at the dose of 50 and 100 mg/kg respectively.

Table 4. Animal Groupings

Groups	Drug Treatment
I	Control (1ml/kg, p.o)
II	Poonaga parpam (50mg/kg, p.o)
III	Poonaga parpam (100mg/kg, p.o)

Numbering and Identification

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table-5 Group Numbering and Identification animals were marking on body

Group No	Animal marking
Control	H,B,T, HB,BT,HT
Low dose	H,B,T,HB,BT,HT
High dose	H,B,T,HB,BT,HT

H-head, B-body, T-tail

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage . label and body marking on the animals:

Table-6. Numbering and Identification cage label and body marking on the animals.

Cage no	Group no	Animal marking	Sex
1	Control	H,B,T	Male
		HB,BT,HT	Female
2	Low dose	H,B,T	Male
		HB,BT,HT	Female
3	High dose	H,B,T	Male
		HB,BT,HT	Female

HB – Head Body, BT – Body Tail, HT – Head Tail

The vehicle (Honey) and test drugs were administered orally, once daily for 28 days. Body weight, food intake and water intake were monitored at regular intervals. The animals were sacrificed on 29th day for biochemical and histopathological studies. Prior to the sacrifice, animals were isolated in individual cages and fasted for 12 hr, with water provided *ad libitum*.

Then, they were anaesthetized with pentobarbitone (45mg/kg, i.p) and the blood was collected by sino-orbital puncture. Blood samples for the determinations of hematological parameters (Ghai, 1995) were collected in heparinized tubes and used for the following determinations, hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count and differential count (DC)

Non-heparinized tubes were used for serum biochemistry determinations. To obtain the serum, blood samples were placed at room temperature for approximately 30 min. Then, the tubes were centrifuged at 3000 x *g* for 10 min and the supernatants were taken for the determinations of SGPT (AST), ALT (SGOT), ALP, Creatinine, Blood urea nitrogen, Creatinine Phosphokinase and Lactate Dehydrogenase.

Estimation of AST and ALT

Activities of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) were assayed by the method of Reitman and Frankel, 1957. 0.2 ml of serum with 1 ml of substrate (aspartate and α-ketoglutarate for AST; alanine and α -keto glutarate for ALT, in phosphate buffer pH 7.4) was incubated for an hour in case of

AST and 30 minutes for ALT. 1 ml of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 ml of 0.4N NaOH was added and absorbance was read at 540 nm. Activities expressed as IU/L.

Estimation of ALP (King, 1965)

Set up three test-tubes. Into the 1st (test), added 5 ml of the substrate solution (p-nitrophenyl phosphate in glycine/NaOH buffer) followed by 0.1 ml of serum. After 30 minutes reaction at 37^0C, the optical density was measured at 405 nm. Into the 2nd tube, 5 ml of substrate solution was added with 0.1 ml of serum. After mixing, the optical density was measured immediately. Into the 3rd tube, 0.1 ml of water was added with 5.0 ml of p – nitrophenol standard solution, optical density was measured.

Estimation of Blood Urea (Natelson et al., 1951)

Labeled three test-tubes as B, T and S. Into B, pipette, 0.02 ml water, into T, 0.02 ml blood and into S, 0.02 ml standard urea solution (40 mg urea in 100 ml of water). 0.1 ml of diacetyl monoxime solution and 5 ml of acid regent (Thiosemicarbazide) was added into all the test-tubes. Mixed and kept in a boiling water bath for 15 minutes. After cooling, the absorbance was read at 540 nm and concentration of urea in mg/dl was calculated.

Estimation of Serum Creatinine (Slot, 1965)

Labeled three test-tubes as B, T and S. Into B, pipetted, 2 ml of water, into T, 2 ml serum and 4 ml of water, into S, 3 ml of water and 1 ml of creatinine standard (4mg/dl). 2 ml of ammonium sulphate and 2 ml of sodium tungstate was added in all the three test-tubes. Centrifuged and removed 3 ml of supernatant from each test tube. 1 ml of picric acid and distilled water was added to the supernatant of test tubes B, T and S. Absorbance was read at 520 nm and concentration of serum creatinine in mg/dl was calculated.

Determination of Creatine Phosphokinase

The activity of CK was estimated by the method of Rosalki (1967). CK catalyses the conversion of creatine phosphate and ADP to creatine and ATP. The ATP and glucose are converted to ADP and glucose-6-phosphate by hexokinase (HK). Glucose -6-phosphate dehydrogense (G-6-PDH) oxidizes D-glucose-6-phosphate and reduces the nicotinamide adenine dinucleotide (NAD). The rate of NADH formation, measured at 340nm, is directly proportional to serum CK activity. One ml of working reagent was added to 50 µl of test sample, mixed and incubated at 37^o C for 1min. After incubation, change in the optical density was measured for 3

min at an interval of 1min against blank at 340nm. The activity of creatine Phosphokinase was expressed as U/L.

Determination of Lactate Dehydrogenase

The activity of LDH was estimated by the method of Teitz,1976. The enzyme LDH is distributed in tissues particularly in heart, muscle and kidney. LDH catalyzes the oxidation of lactate to pyruvte in the presence of NAD which is subsequently reduced to NADH. The rate of NADH formation was measured at 340nm and is directly proportional to LDH activity. One ml of the working reagent was added to 10 µl of test sample, mixed and incubated at 37^{o} C for 1min. After incubation, change in the optical density was measured for 1min at an interval of 1 min against reagent blank at 340 nm. The activity of LDH was expressed as U/L.

After blood collection, the animals were sacrificed by cervical decapitation and the organs such as brain, heart, liver, spleen, kidney and testis were removed and weighed. The organs were preserved in 10% buffered formaldehyde for histopathological observations.

HISTOPATHOLOGICAL STUDIES

Anatomy of the liver was studied immediately after sacrificing the animals. A small portion was fixed in 10% neutral buffered formalin as described by Luna 14. Thin sections of 4-5µm were taken, stained with Haematoxylin and Eosin and histology was studied.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's *'t'* – test using graph pad version I. *P* values <0.05 were considered significantly.

7. RESULT AND INFERENCES

QUALITATIVE AND QUANTITATIVE ANALYSIS

Table -7

Colour characters of *POONAGA PARPAM*

S.No	Nature of drug	Nature of colour
1	***POONAGA PARPAM***	Sensaayal

Table 8— Physicochemical analysis of samples of *POONAGA PARPAM*

[Values are mean of three determinations ±SEM]

Parameters	Total ash	Values
Ash value	Water soluble ash	8.65±0.011
	Acid insoluble ash	0.75±0.011
Loss on drying	Loss on drying at 70°C	7.20±0.240

SEM- singularity expansion method

Table-9

Particle size and pH of *POONAGA PARPAM*

S.No	Parameters	Values obtained
1	Particle size by SEM	0.5-2 μ
2	pH	8.840

PHYTOCHEMICAL ANALYSIS

PHYTO-CHEMICAL STUDY OF POONAGA PARPAM

This experimental study was taken up to qualitative analysis of Phyto-chemicals in the drug of **Poonaga Parpam** using various test and the results are exhibited in Table No.10

Table No 10: Incidence of various phyto-chemicals in Poonaga Parpam

S.No.	Name of Tests Conducted	Result Observed
Observation of Alkaloids		
1.	Mayer's Test	Negative
2.	Dragendroff's Test	Negative
3.	Hager's Test	Negative
Observation of Carbohydrates and Glycosides		
4.	Molisch Test	Positive
5.	Legal's Test	Positive
6.	Borntrager's Test for anthraquinones	Positive
Observation of Phytosterols		
7.	Liebermann – Burchard Test	Negative
8.	Salkowski Test	Negative
Observation of Flavanoids		
9.	Shinoda Test (Magnesium turnings & Hydrochloric acid)	Negative
10.	Fluorescence Test	Negative
Observation of Tannins		
11.	Ferric chloride test	Negative
12.	Potassium dichromate test	Negative

13.	Lead acetate test	Positive
14.	Millon's test	Negative
15.	Biuret test	Positive
16.	Ninhydrin test	Negative
Observation of fixed oils and fats		
17.	Spot test	Negative
18.	Saponification test	Negative
Observation of Lignin		
19.	Phloroglucinol test	Negative
Observation of Saponins		
20.	Frothing test	Negative

Note: *Positive indicates the presence of Phytochemical; Negative indicates the absence of Phytochemical*

Result :

Phytochemical analysis of poonaga Parpam shows the presence of carbohydrates, glycosides and tannins.

FOURIER TRANSFORM - INFRARED SPECTROSCOPY

IR TRACER - 100 THE NEW FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER

Chart- 1 FTIR results of Poonaga Parpam

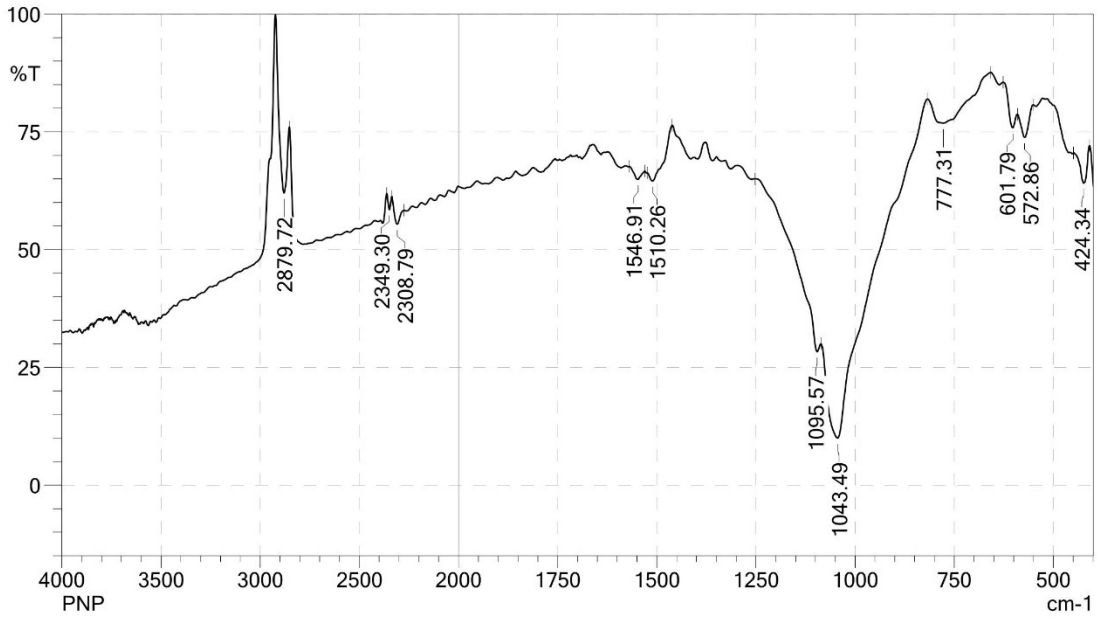


Table – 11 Functional group by FTIR study

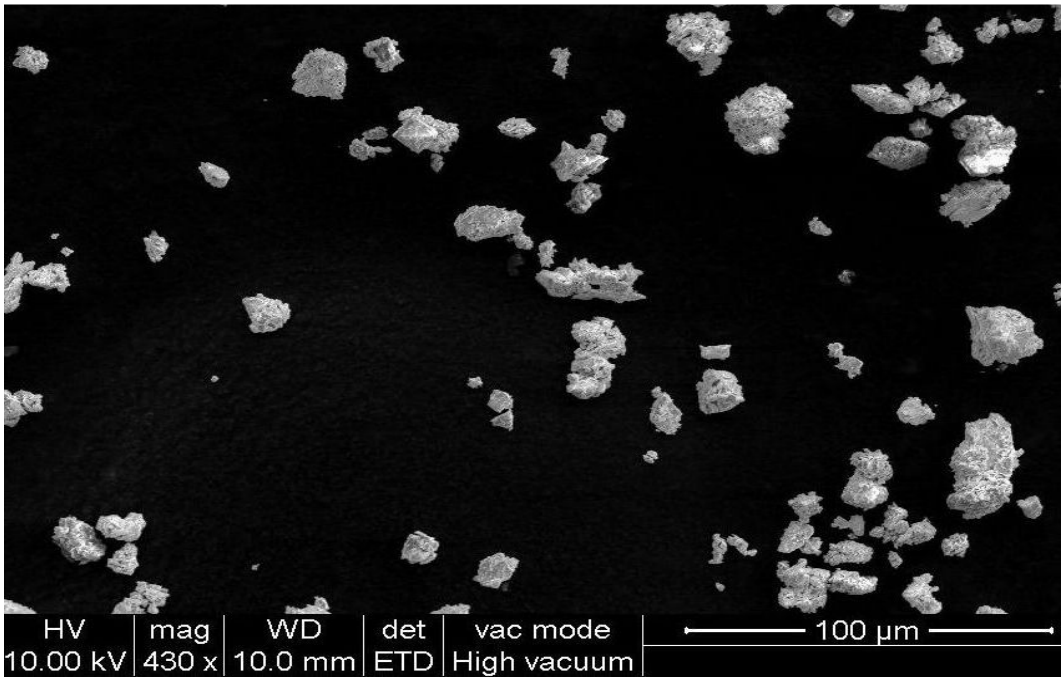
FREQUENCY cm-1	BOND	FUNCTIONAL GROUP
424.34(M)	C-I stretch	Halo Compounds
572.86(M)	C-I stretch	Halo Compounds
601.79(M)	C-Br stretch	Alkyl Halides
777.31(M)	C-CI stretch	Alkyl Halides
1043.49(M)	C-N stretch	Aliphatic amines
1095.57(M)	C-N stretch	Aliphatic amines
1510.26(S)	N-O asymmetric stretch	Nitro Compounds
1546.91(S)	N-O asymmetric stretch	Nitro Compounds
2308.79(S)	O=C=O stretch	Carbon dioxide
2349.30(S)	O=C=O stretch	Carbon dioxide
2879.72(M)	C-H stretch	Alkane

S-strong, W-weak, M-medium

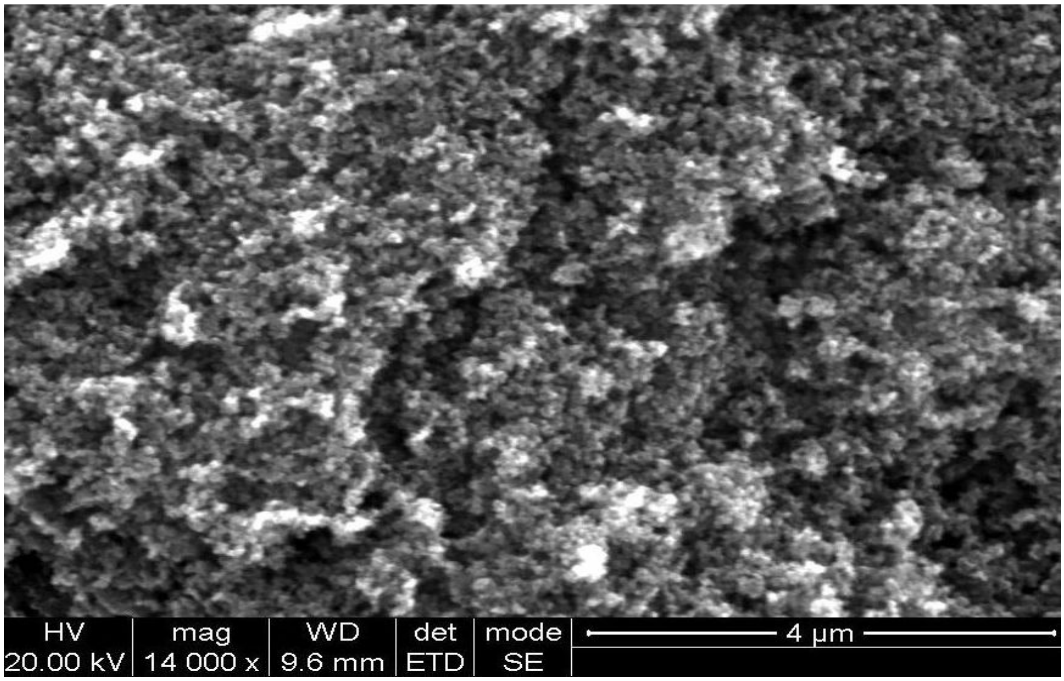
Result: FTIR study of Poonaga Parpam shows the presene of functional groups such as Halo compounds, Alkyle halides, Aliphatic amines, Nitro compounds, Carbon dioxide and Alkane.

SEM ANALYSIS

Scanning Electron Microscope (SEM)



SEM -7000 Magnification



SEM -14000 Magnification

Result:

The particles were stabilized and have irregular morphology. The particles were distributed in range 100µm and the size is below 4 µm

ICP – OES of POONAGA PARPAM

ICP – OES of PP

S. No	Elements	Wavelength (nm)	Concentration
1.	Al	396.152	BDL
2.	As	188.979	BDL
3.	Ca	315.807	**02.080mg/L**
4.	Cd	228.802	BDL
5.	Cu	327.393	82.561mg/L
6.	Fe	238.204	01.306mg/L
7.	Hg	253.652	BDL
8.	K	766.491	**03.021mg/L**
9.	Mg	285.213	**01.204mg/L**
10.	Na	589.592	**01.300mg/L**
11.	Ni	231.604	BDL
12.	Pb	220.353	BDL
13.	P	213.617	**196.381mg/L**

BDL: Below Detectable Limit(Normal-1ppm)

1% = 10000ppm,

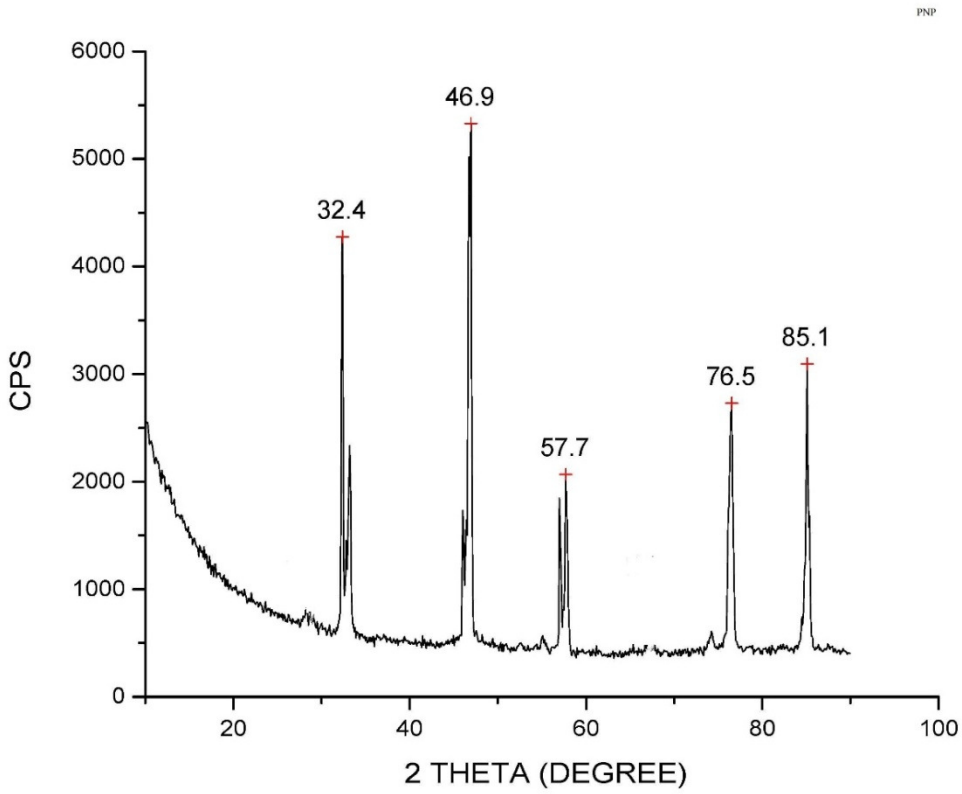
1ppm = 1/1000000 or 0.0001%

Results:

The result indicate that the formulation is extremely safe as it contains heavy metals within specified limits.

It also has physiologically important minerals like Calcium, Copper, Ferrous,Magnesium, Sodium, Phosphorus and Potassium. In *Poonaga Parpam ,* the heavy metals like Arsenic, Mercury, Lead, Cadmium and trace element like Nikkal were below detectable level. This reveals the safety of the drug and it has free from toxic substances and has no side effects.

XRD of POONAGA PARPAM



Result:

These XRD fingerprints shows both the similarities and differences of the sample successfully and is a valuable primary tool for checking the quality control of herbo animal medicines. Modern techniques are necessary to standardize and bringout high quality herbal products owing their complex nature. The different peaks shows the presence of minerals in the sample.

BIO-CHEMICAL ANALYSIS OF *POONAGA PARPAM*

Preparation of the extract:

100mgs of parpam is weighted accurately and placed into a clean beaker and added a few drops of Conc. Hydrochloric acid and evaporated it well. After evaporation cooled the content and added a few drops of conc. nitric acid and evaporated it well. After cooling the content add 20ml of distilled water and dissolved it well. Then it is transferred to 100ml volumetric flask and made up to 100ml with distilled water, mix well, filter it. Then it is taken for analysis.

Table No. 12

S.NO	EXPERIMENTS	OBSERVATION	INFERENCE
1.	**Test for calcium:** 2 ml 0f the above prepared extract taken in a clean test tube to this add 2 ml of 4% ammonium oxalate solution.	Formation of white colour precipitate	presence of calcium
2.	**Test for sulphate:** 2 ml of the extract is added to 5% barrium chloride solution.	Formation of white colour precipitate	Presence of sulphate.
3.	**Test for chloride:** The extract is treated with silver nitrate solution.	Formation of white colour precipitate	Presence of chloride.
4.	**Test for carbonate:** The substance is treated with concentrated HCL.	No brisk effervescence is formed.	Absence of carbonate.
5.	**Test for starch:** The extract is added with weak iodine solution.	No blue colour is formed	Absence of starch.

6.	**Test for ferric iron:** The extract is acidified with glacial acetic acid and potassium ferro cyanide.	No blue colour is formed	Absence of ferric iron.
7.	**Test for ferrous iron:** The extract is treated with concentrated nitric acid ammonium thiocyanide solution.	Appearance of blood red colour.	presence of ferrous iron
8.	**Test for phosphate:** The extract is treated with ammonium molybdate and concentrated nitric acid.	Formation of yellow precipitate	presence of phosphate.
9.	**Test for albumin:** The extract is treated with esbach's reagent.	No yellow precipitate is formed	Absence of albumin.
10.	**Test for tannic acid:** The extract is treated with ferric chloride.	No blue black precipitate is formed	Absence of tannic acid.
11.	**Test for unsaturation:** Potassium permanganate solution is added to the extract.	It does not get decolourised.	Absence of unsaturated compounds.
12.	**Test for the reducing sugar:** 5ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minutes.	No Colour change occurs.	Absence of reducing sugar.
13.	**Test for amino acid:** One or two drops of the extract is placed on a filter paper and dried well. After drying, 1 % ninhydrin is sprayed over the same and dried it well.	No Violet colour is formed	Absence of amino acid.

14.	**Test for zinc:** The extract is treated with potassium ferro cyanide.	No white precipitate is formed	Absence of zinc.

Inference:

The Biochemial analysis of ***POONAGA PARPAM*** indicates the presence of calcium, sulphate, chloride, ferrous iron and phosphate.

Biochemical Analyis report was given by **Mrs. N.Nagaprema, M.Sc., H.O.D, Bio Chemical Department, Government Siddha Medical College, Palayamkottai.**

ACUTE TOXICITY STUDY ON POONAGA PARPAM

Effect of Acute Toxicity (14 Days) of *Poonaga Parpam*

Table 13 Physical and behavioral examinations.

Group no.	Dose(mg/kg)	Observation sign	Mortality.
Group-I	Control	Normal	0 of 3
Group- II	5 mg/kg	Normal	0 of 3
Group-III	50 mg/kg	Normal	0 of 3
Group-IV	300 mg/kg	Normal	0 of 3
Group-V	2000 mg/kg	Normal	0 of 3

Table – 14 shows the effect of control – distilled water (1ml/kg) on general behavior after single oral administration in Rat.

Sl .No	General Behavior	Time of observation after control – distilled water (1ml/kg) administration			
		1st hour	3rd hour	4th hour	24th hour
1	Sedation	-	-	-	-
2	Hypnosis	-	-	-	-
3	Convulsion	-	-	-	-
4	Ptosis	-	-	-	-
5	Analgesia	-	-	-	-
6	Stupor reaction	-	-	-	-
7	Motor activity	-	-	-	-
8	Musle relaxant	-	-	-	-
9	CNS stimulant	-	-	-	-
10	CNS depressant	-	-	-	-
11	Pilo erection	-	-	-	-
12	Skin colour changes	-	-	-	-
13	Lacrimation	-	-	-	-
14	Stool consisteny	-	-	-	-

+ Present, - Absent

Table - 15 shows the effect of Poonaga Parpam (5mg/kg) on general behavior after single oral administration in Rat.

Sl.No	General behaviour	Time of observation after Poonaga Parpam (5 mg / kg) administration			
		1st hour	3rd hour	4th hour	24th hour
1	Sedation	-	-	-	-
2	Hypnosis	-	-	-	-
3	Convulsion	-	-	-	-
4	Ptosis	-	-	-	-
5	Analgesia	-	-	-	-
6	Stupor reaction	-	-	-	-
7	Motor activity	-	-	-	-
8	Musle relaxant	-	-	-	-
9	CNS stimulant	-	-	-	-
10	CNS depressant	-	-	-	-
11	Pilo erection	-	-	-	-
12	Skin colour changes	-	-	-	-
13	Lacrimation	-	-	-	-
14	Stool consisteny	-	-	-	-

+ Present, - Absent

Table-16 shows the effect of Poonaga Parpam (50mg/kg) on general behavior after single oral administration in Rat.

Sl.No	General behaviour	Time of observation after Poonaga Parpam (50 mg / kg) administration			
		1st hour	3rd hour	4th hour	24th hour
1	Sedation	-	-	-	-
2	Hypnosis	-	-	-	-
3	Convulsion	-	-	-	-
4	Ptosis	-	-	-	-
5	Analgesia	-	-	-	-
6	Stupor reaction	-	-	-	-
7	Motor activity	-	-	-	-
8	Musle relaxant	-	-	-	-
9	CNS stimulant	-	-	-	-
10	CNS depressant	-	-	-	-
11	Pilo erection	-	-	-	-
12	Skin colour changes	-	-	-	-
13	Lacrimation	-	-	-	-
14	Stool consisteny	-	-	-	-

+ Present, - Absent

Table-17 shows the effect of Poonaga Parpam (300mg/kg) on general behavior after single oral administration in Rat.

Sl.No	General behaviour	Time of observation after Poonaga Parpam (300 mg / kg) administration			
		1st hour	3rd hour	4th hour	24th hour
1	Sedation	-	-	-	-
2	Hypnosis	-	-	-	-
3	Convulsion	-	-	-	-
4	Ptosis	-	-	-	-
5	Analgesia	-	-	-	-
6	Stupor reaction	-	-	-	-
7	Motor activity	-	-	-	-
8	Musle relaxant	-	-	-	-
9	CNS stimulant	-	-	-	-
10	CNS depressant	-	-	-	-
11	Pilo erection	-	-	-	-
12	Skin colour changes	-	-	-	-
13	Lacrimation	-	-	-	-
14	Stool consisteny	-	-	-	-

+ Present, - Absent

Table-18 shows the effect of Poonaga Parpam (2000 mg/kg) on general behavior after single oral administration in Rat.

Sl.No	General behaviour	Time of observation after Poonaga Parpam (2000 mg / kg) administration			
		1st hour	3rd hour	4th hour	24th hour
1	Sedation	-	-	-	-
2	Hypnosis	-	-	-	-
3	Convulsion	-	-	-	-
4	Ptosis	-	-	-	-
5	Analgesia	-	-	-	-
6	Stupor reaction	-	-	-	-
7	Motor activity	-	-	-	-
8	Musle relaxant	-	-	-	-
9	CNS stimulant	-	-	-	-
10	CNS depressant	-	-	-	-
11	Pilo erection	-	-	-	-
12	Skin colour changes	-	-	-	-
13	Lacrimation	-	-	-	-
14	Stool consisteny	-	-	-	-

+ Present, - Absent

Table-19 Home cage activity

Functional and Behavioral observation	Observation	5mg/kg Group (G-I)	50mg/kg (G-II)	300mg/kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Body position	Normal	3	3	3	3	3
Respiration	Normal	3	3	3	3	3
Clonic involuntary Movement	Normal behaviour	3	3	3	3	3
Tonic involuntary Movement	Normal behaviour	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

Table-20 Hand held observation

Functional and Behavioral Observation	Observation	Control	5 mg/ kg (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	2000 mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal behaviour	3	3	3	3	3	3
Lacrimation	Normal behaviour	3	3	3	3	3	3
Salivation	Normal behaviour	3	3	3	3	3	3
Piloerection	Normal behaviour	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

Table-21 Mortality

Group No	Dose No (mg/kg)	Mortality
Group-I	Control	0 of 3
Group-II	5 (mg/kg)	0 of 3
Group-III	50 (mg/kg)	0 of 3
Group-IV	300 (mg/kg)	0 of 3
Group-V	2000(mg/kg)	0 of 3

The results of acute toxicity study of Poonaga parpam was shown on table 15. The Poonaga parpam did not alter the general behavior after 1hour to 24hours of oral administration. There was no mortality with the Poonaga parpam after 1hr to 24 hrs even at higher dose of 2000mg/kg. It did not show any lethality or toxic reactions during and after the study. From the doses administered in acute toxicity study, their 2 doses 50 and 100 mg/kg were selected for sub-acute toxicity study.

SUB-ACUTE TOXICITY STUDY

Table 22. Effect of Poonaga parpam on body weight during 28 days drug administration in rats

Groups	Drug Treatment	Body Weight (gms)				
		1st Day	7th Day	14th Day	21st Day	28th Day
I	Control – Distilled water (1ml/kg, p.o)	168.73± 3.42	176.22± 4.97	196.34± 4.77	210.54± 6.04	220.22± 4.40
II	**Poonaga parpam** (50mg/kg, p.o)	193.33± 16.46	203.33± 17.20	201.66± 24.98	203.33± 20.72	195.83± 21.5
III	**Poonaga parpam** (100mg/kg, p.o)	215± 17.41	219.16± 21.63	210.83± 25.14	210± 23.38	209.16± 21.73

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 1. Effect of Poonaga parpam on body weight during 28 days drug administration in rats

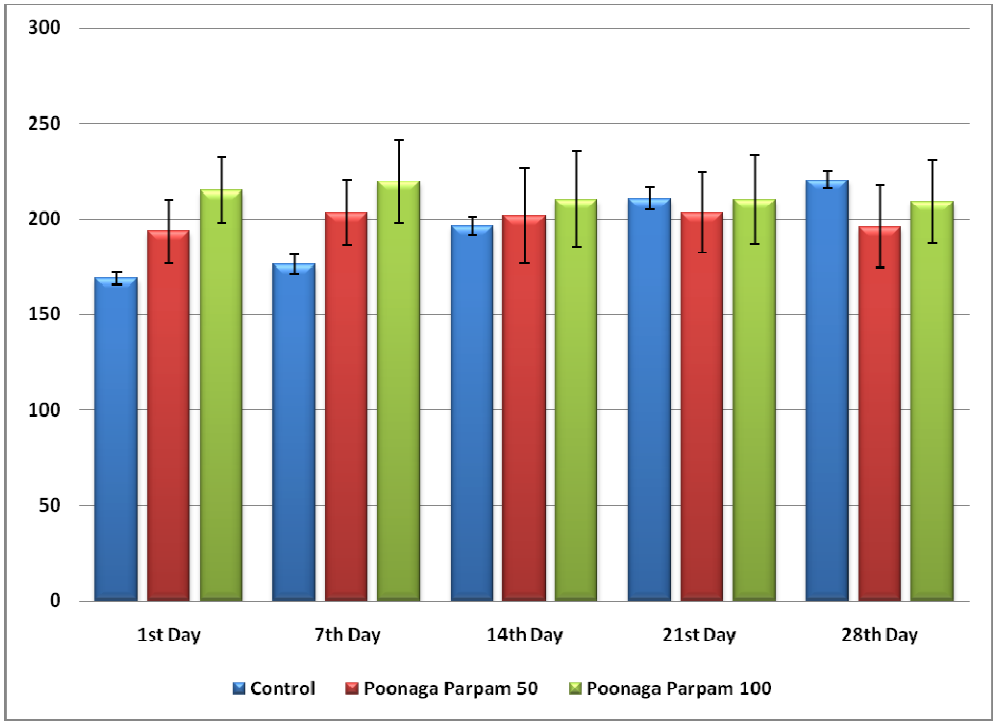


Table 23. Effect of Poonaga parpam on food intake during 28 days drug administration in rats

Groups	Drug Treatment	Food Intake (gms)				
		1st Day	7th Day	14th Day	21st Day	28th Day
I	Control - Distilled water (1ml/kg, p.o)	38.42±1.32	42.56±2.86	39.30±2.20	53.75±3.19	56.90±2.72
II	**Poonaga parpam** (50mg/kg, p.o)	60.42±3.77	68.54±2.90	65.00±3.72	45.64±2.20	50.80±3.66
III	**Poonaga parpam** (100mg/kg, p.o)	65.74±2.60	69.87±3.33	69.50±3.05	70.60±3.75	75.88±3.26

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 2. Effect of Poonaga parpam on food intake during 28 days drug administration in rats

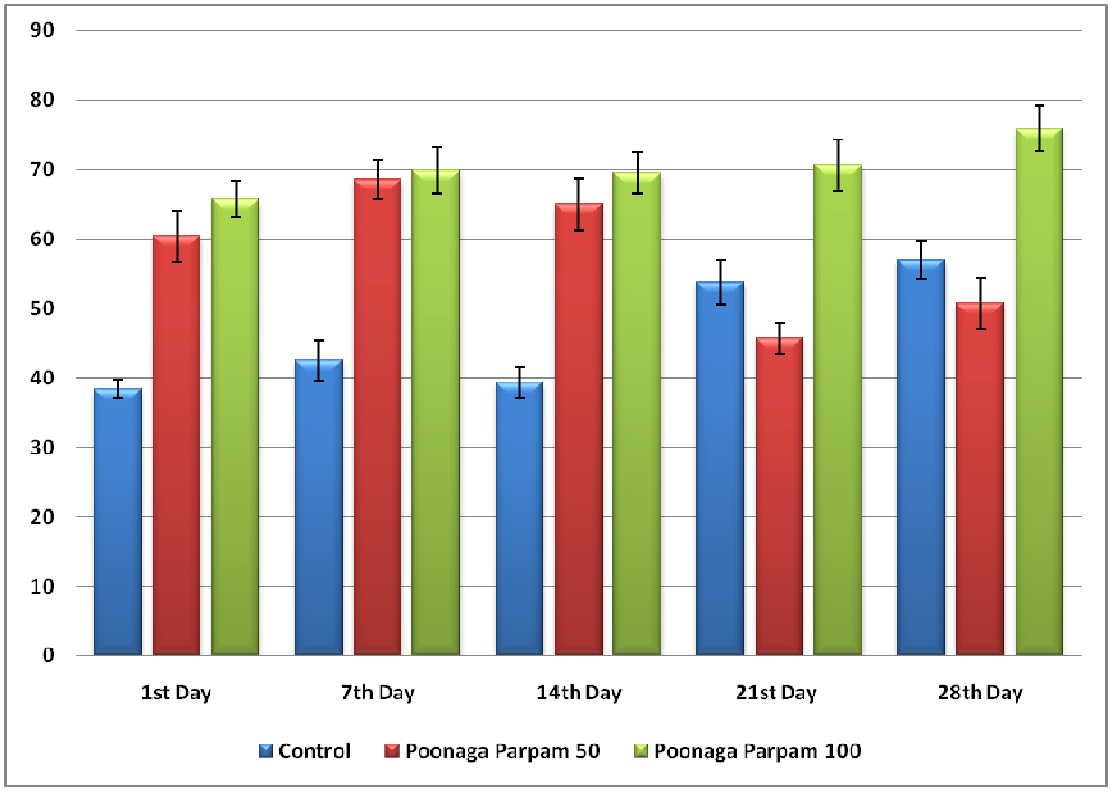


Table 24. Effect of Poonaga parpam on water intake during 28 days drug administration in rats

Groups	Drug Treatment	Water Intake (ml)				
		1st Day	7th Day	14th Day	21st Day	28th Day
I	Control - Distilled water (1ml/kg, p.o)	51.66± 2.02	58.76± 4.22	64.00± 3.90	70.34± 4.73	65.76± 4.80
II	**Poonaga parpam** (50mg/kg, p.o)	63.20± 3.20	72.53± 3.25	67.35± 2.52	77.72± 3.24	75.80± 3.27
III	**Poonaga parpam** (100mg/kg, p.o)	85.49± 4.75	67.35± 3.60	67.35± 4.77	72.53± 4.54	77.72± 6.24

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 3. Effect of Poonaga parpam on water intake during 28 days drug administration in rats

Table 25. Shows the effect of Poonaga parpam on RBC, WBC and Hb in rats after 28 days drug administration

Groups	Drug Treatment	RBC million cells/cmm	WBC cells/cmm	Haemoglobin gm %
I	Control – Distilled water (1ml/kg, p.o)	4.57 ± 0.16	8638.52± 87.66	11.77± 0.28
II	**Poonaga parpam** (50mg/kg, p.o)	4.53 ± 0.23	8355.83± 136.48	11.80± 0.30
III	**Poonaga parpam** (100mg/kg, p.o)	4.77 ± 0.18	9317.47± 132.20	11.56± 0.47

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 4. Shows the effect of Poonaga parpam on RBC and Hb in rats after 28 days drug administration

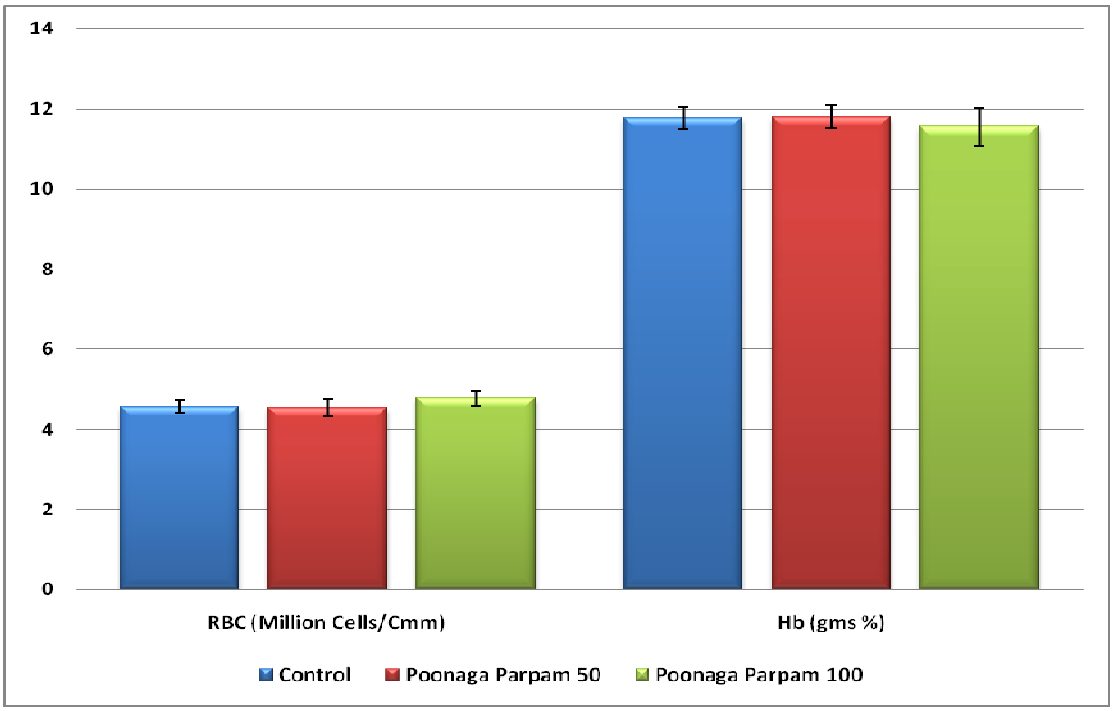
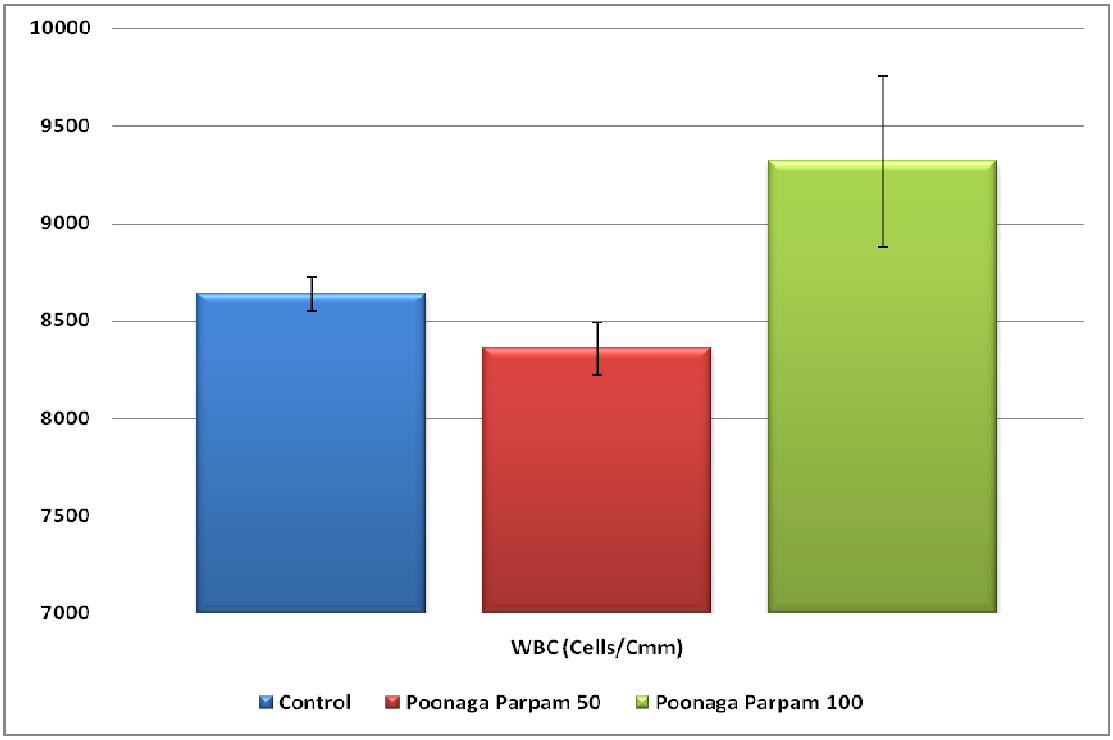


Figure 5. Shows the effect of Poonaga parpam on WBC in rats after 28 days drug administration



Shows the effect of Poonaga parpam on Differential Count in rats after 28 days drug administration

Groups	Drug Treatment	Differential Count %			
		Neutophils	***Eosinophils***	***Monocyte***	***Lympocyte***
I	Control - Distilled water (1ml/kg, p.o)	30.00± 1.79	1.17± 0.31	3.33± 0.42	65.83± 1.64
II	**Poonaga parpam** (50mg/kg, p.o)	28.20± 0.49	1.20± 0.20	2.80± 0.37	68.80± 2.42
III	**Poonaga parpam** (100mg/kg, p.o)	29.60± 1.44	1.60± 0.24	3.60± 0.40	66.60± 1.17

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 6. Shows the effect of Poonaga parpam on Differential Counts in rats after 28 days drug administration

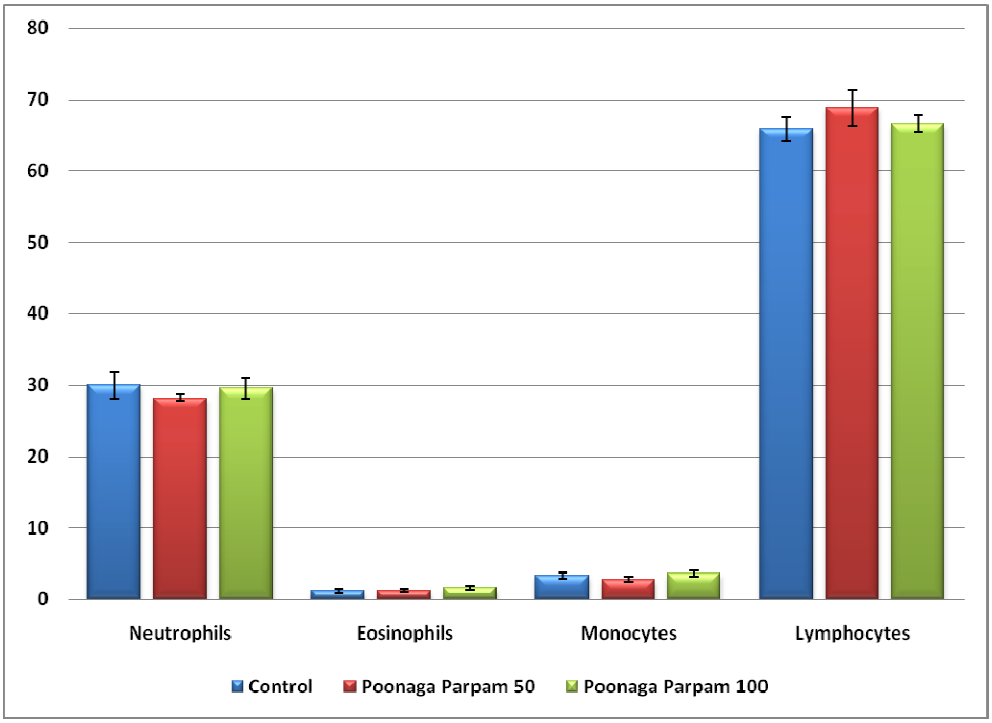


Table 26. Shows the effect of Poonaga parpam on Hepatic Functions (SGPT, SGOT and ALP) in rats after 28 days drug administration

Groups	Drug Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
I	Control - Distilled water (1ml/kg, p.o)	83.83± 1.42	150.17± 4.59	270.83± 4.17
II	**Poonaga parpam** (50mg/kg, p.o)	88.20± 2.71	152.00± 3.83	273.80± 3.53
III	**Poonaga parpam** (100mg/kg, p.o)	110.40± 3.89	177.60± 3.88	290.20± 3.39

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 7. Shows the effect of Poonaga parpam on Hepatic Functions in rats after 28 days drug administration

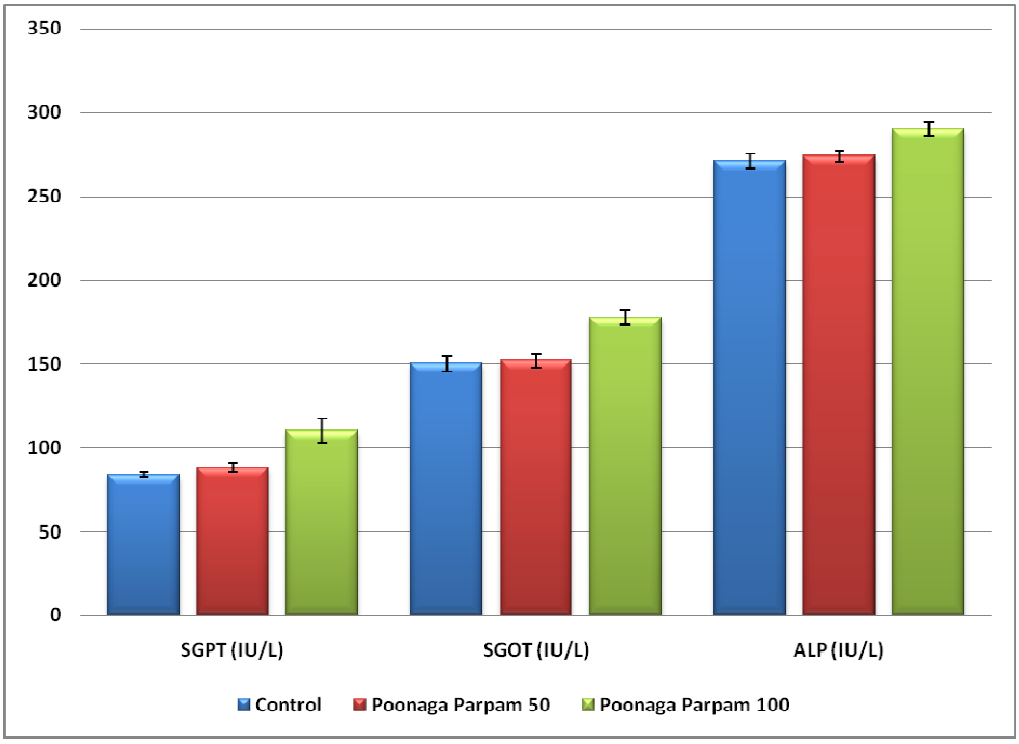


Table Shows the effect of Poonaga parpam on Kidney Functions in rats after 28 daysdrug administration

Groups	Drug Treatment	Urea (mg/dl)	Creatinine (mg/dl)
I	Control - Distilled water (1ml/kg, p.o)	34.67± 1.12	0.84± 0.07
II	**Poonaga parpam** (50mg/kg, p.o)	37.00± 3.21	1.07± 0.15
III	**Poonaga parpam** (100mg/kg, p.o)	61.80± 1.88	1.63± 0.29

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 8. Shows the effect of Poonaga parpam on Kidney Functions (Blood Urea and Creatinine) in rats after 28 days drug administration

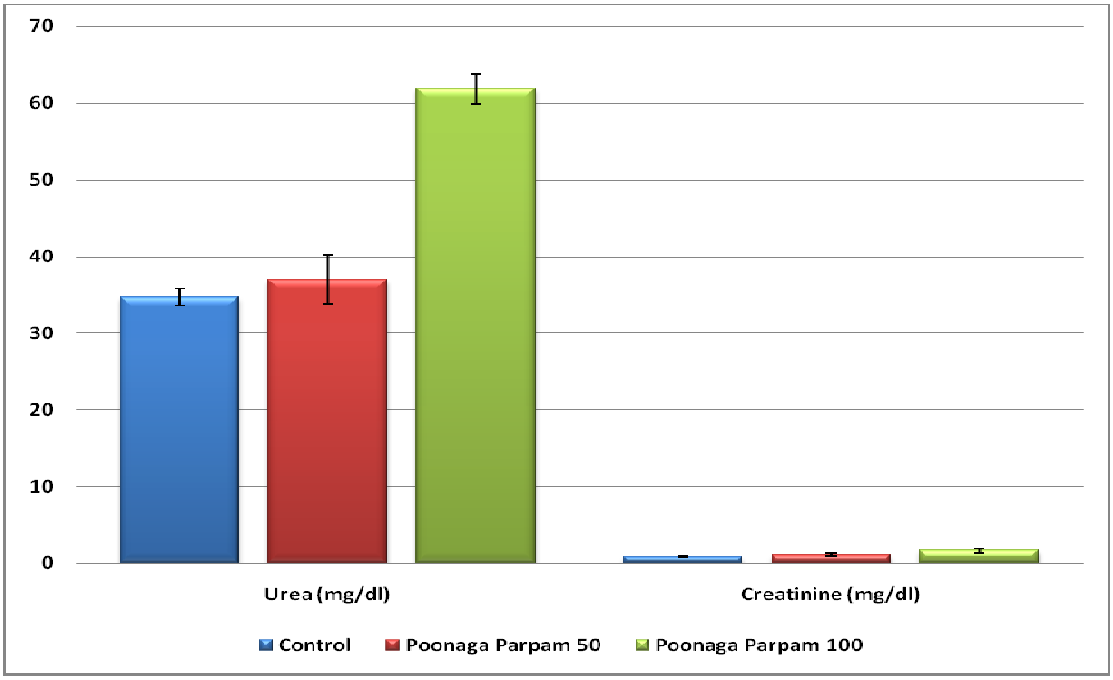


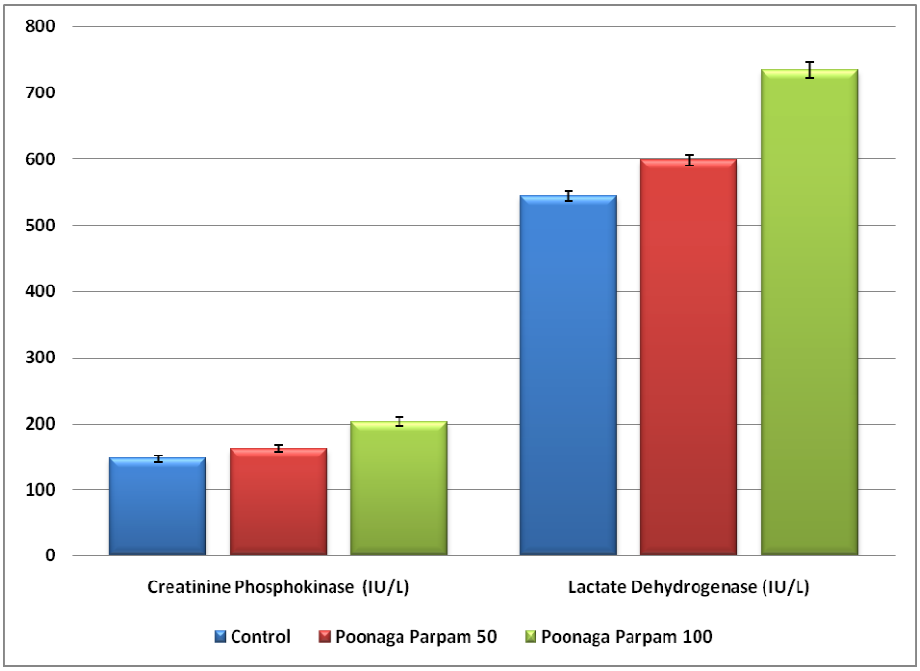
Table Shows the effect of Poonaga parpam on Cardiac Functions in rats after 28 days drug administration

Groups	Drug Treatment	Creatinine Phosphokinase (IU/L)	Lactate Dehydrogenase (IU/L)
I	Control - Distilled water (1ml/kg, p.o)	146.83± 4.79	543.50± 7.72
II	**Poonaga parpam** (50mg/kg, p.o)	162.20± 4.75	597.80± 8.21
III	**Poonaga parpam** (100mg/kg, p.o)	203.20± 4.38	734.40± 7.89

Values are in mean ± SEM (n=6)

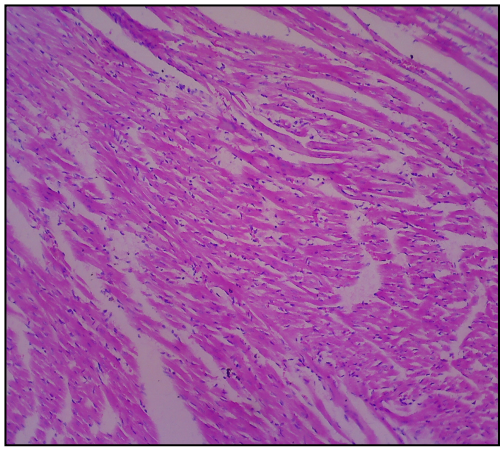
*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 9. Shows the effect of Poonaga parpam on Cardiac Functions (Creatinie Phosphokinase and Lactate Dehydrogenase and in rats after 28 days drug administration

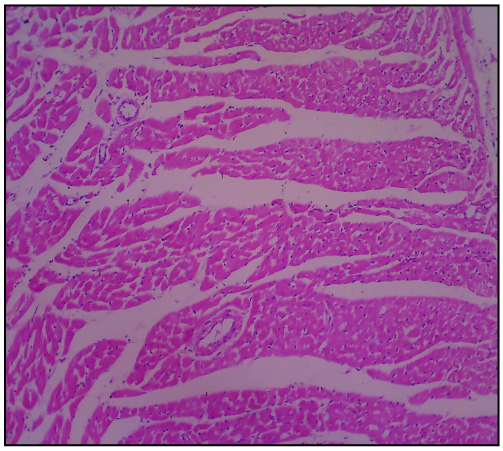


HISTOPATHOLOGICAL STUDIES

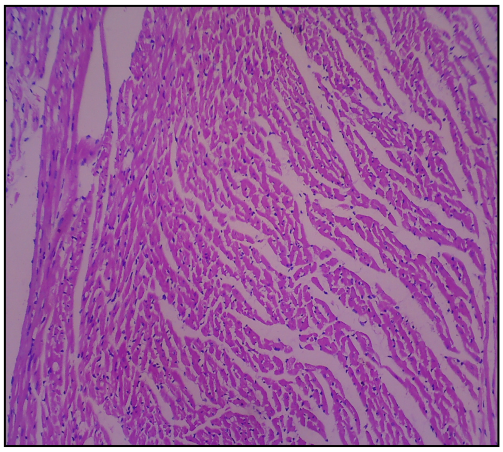
HEART



CONTROL



LOW DOSE (50mg/kg)

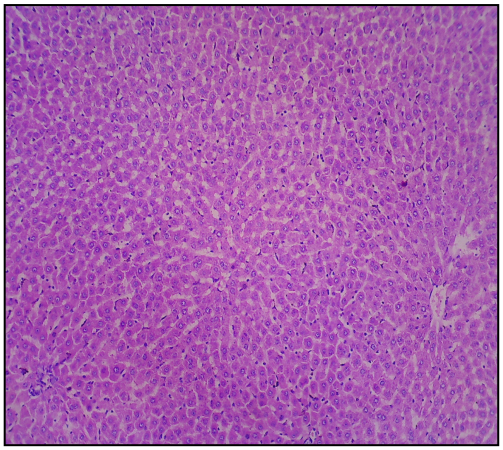


HIGH DOSE (100mg/kg)

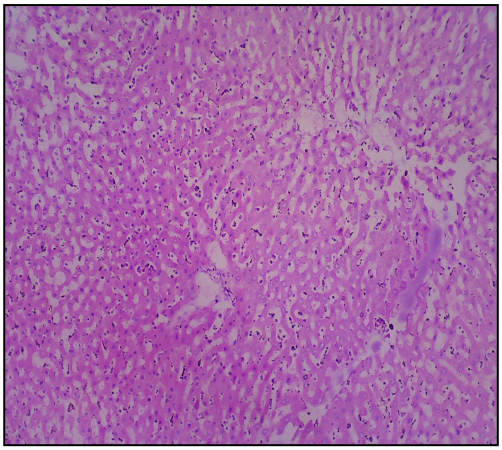
RESULT :

Normal cardiac muscle fibers seen

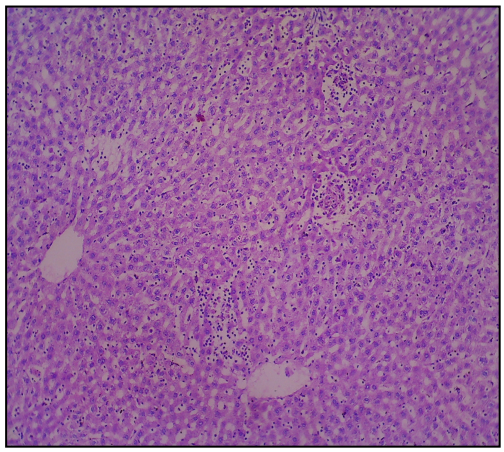
LIVER



CONTROL



LOW DOSE (50mg/kg)

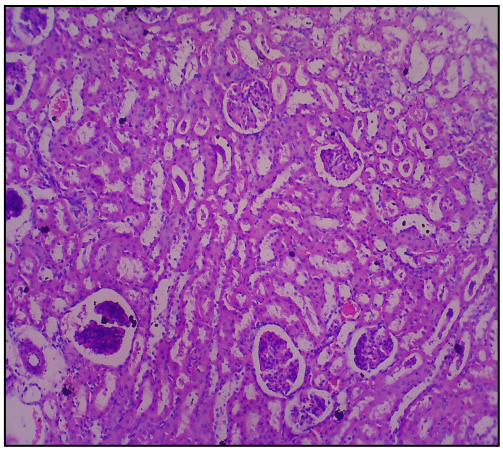


HIGH DOSE (100mg/kg)

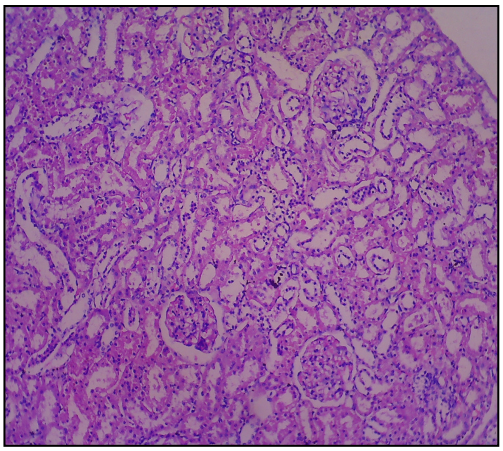
RESULT :

Normal Liver parenchyma seen

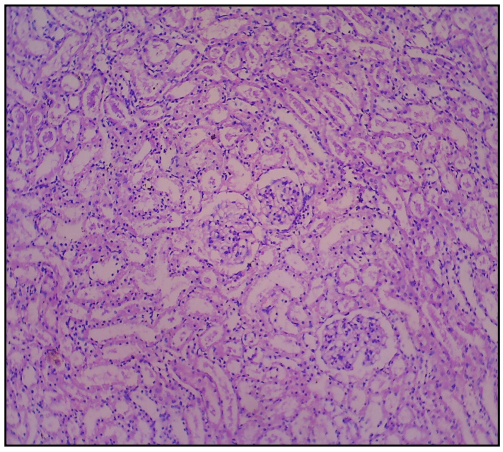
KIDNEY



CONTROL



LOW DOSE (50mg/kg)



HIGH DOSE (100mg/kg)

RESULT :

Normal Renal parenchyma seen

RESULT

In sub-acute toxicity study, body weight, food intake and water intake were observed on 1st, 7th, 14th 21st and 28th day of Poonaga parpam after drug administration. The effect of Poonaga parpam regarding body weight during 28 days treatment in rats was given in table 19 and as figure 1. There was no significant change in the body weight compared to control with all the two doses of Poonaga parpam during 28 days treatment.

The effect of Poonaga parpam on food intake during 28 days treatment in rats was given in table 19 and figure 2. Poonaga parpam did not alter the food intake at both the dose levels as compared to control during the 28 days treatment. It indicates that it does not influence food intake.

The effect of Poonaga parpam on water intake during 28 days treatment in rats was given in table 21 and figure 3. Poonaga parpam did not alter the water intake at both the dose levels as compared to control during the 28 days treatment.

Table 22, figure 4 and 5, shows the effect of Poonaga parpam on haematological parameters like RBC, WBC and Hb in rats after 28 days treatment. Both the doses of Poonaga parpam did not produce any significant change in RBC and Hb compared to control. Poonaga parpam at high dose (100mg/kg) significantly ($P<0.05$) increase the WBC count as compare to control but low dose (50mg/kg) did not show any significant change in WBC count.

The effect of Poonaga parpam on Differential Count in rats after 28 days treatment was shown on table 23 and figure 6. Both the doses of Poonaga parpam did not show any significant change in differential counts like Neutrophils, Eosinpophils, Monocyte and Lympocytes. From the effect of Poonaga parpam on hematological parameters it was found that it does not produce any toxicity in haemopoietic system except mild increase in WBC at high dose.

The blood samples are used to evaluate Hematological parameters (like RBC, WBC, HB and DC) and evaluate biochemical parameters (like SGOT, SGPT, ALP, UREA and CREATININE). The changes in haematological parameters and biochemical parameters are within normal limits.

On completion of the 28days administration of poonaga parpam, Wistar Albino Rats were sacrificed. In macroscopic examination the Heart, Kidneys and Liver organs were weighed. The organs were normal when compared with control group. Histopathological examination revealed normal architecture in comparison with control and treated animal.

In sub-acute toxicity study shows that Poonaga Parpam can be considered safe, as it did not cause either any lethality or adverse changes with general behaviour of rats and also there were no observable detrimental effects on 50mg/kg and 100mg/kg over a period of 28 days. It is concluded that the Poonaga Parpam is relatively safe when administered orally in human for long administration upto the dose 100mg/kg.

BIOSTATISTICAL ASPECTS

Biological assay refers to assessment of the potency of vitamins, hormones, toxicants and drugs of all types by means of the responses produced when doses are given to experimental animals. In every dose response situation, two components must be considered; the stimulus and the subject.

The stimulus is applied to the subject as a started dose namely concentration, weight, time or appropriate measure. The subject manifest a response, the level of intensity below which the response does not occur & above which the response occur, suh a value has often been called threshold. But the term tolerance is now widely accepted.

MEDIAN EFFECTIVE DOSE (E.D.50)

It is the dose which produces the desired response in half the animal population tested.

MEDIAN EFFECTIVE DOSE (E.D.50)

It is the dose which kills half the population of the animal tested.

LD50 Measurement (Toxicity)

- If the test compound shows any pharmacological activity then the L.D.50 of the drug is determined.
- By determining the L.D.50, we can justify whether to proceed with the drug or not.

Table- 27 Acute toxicity study analysis

Group	Dose in mg/kg	No. of rats	No. of rats died
I	Distilled water (1ml/kg)	3	-
II	5	3	-
III	50	3	-
IV	300	3	-
V	2000	3	-

Since there was no mortality of the animal in acute toxicity study, lethal dose of drug could not be calculated.

Table – 28: Sub – Acute Toxicity Analysis

Group	Dose (mg/kg)	No.of rats	Days	No.of rats died
I	Control	6 (3 male, 3 female)	28	-
II	50	6 (3 male, 3 female)	28	-
III	100	6 (3 male, 3 female)	28	-

In case of Sub – Acute Toxicity Study, with the help of physiological parameters such as Haematological investigations and with the Histopathological studies the drug reaction within the animal can be assessed and are being tabulated respectively.

Lethal dose of the drug **"Poonaga Parpam"**can be calculated with higher dose level of the drug which can be done in further studies.

8. DISCUSSION

The preclinical toxicity study of poonaga Parpam was conducted with the prime objective to find out whether the drug has possess any side effects or adverse reactions on long term administration.

Biochemical analysis of Poonaga Parpam indicated the presence of calcium, sulphate, chloride, ferrous iron and phosphate. Heavy toxic metals such as lead, mercury, arsenic, and zinc were absent.

Phytochemical analysis of poonaga Parpam shows the presence of carbohydrates, glycosides and tannins.

FTIR study of Poonaga Parpam shows the presene of functional groups such as Halo compounds, alkyl halides, aliphatic amines, nitro compounds, carbon dioxide and alkanes

Scanned Electron Microscope study of end product shows that the particles were stabilized and have irregular morphology. The particles were distributed in range 100 µm and the size is below 4 µm

In acute toxicity study all the animals were active and did not showed any signs of toxicity. The motor activities were normal in all the 6 groups of animals. This acute toxicity study results reveals that poonaga Parpam was nontoxic upto a dose level of 2000mg/kg body weight of the animal.

Doses for sub-acute toxicity study were selected on the basis of acute toxicity study. The selected doses were 50mg/kg and 100mg/kg body weight of the animal.
In sub-acute toxicity study no signs of toxicity were observed. No changes in the hematological parameters. There was no changes in food intake, water intake and body weight. No mortality occurred till the last day of the study.

Necropsy study of the major organs liver, kidney and heart showed no apparent change in colour. The texture of the organs maintained and the specimens were normal on macroscopical examination when compared with that of the control group.

Histopathological examination revealed normal architecture in comparison with control and treated animal.

Since, there was no mortality in both acute and sub-acute toxicity studies the lethal dose of the drug could not be calculated. The biostatistical analysis reveals that poonaga Parpam is safe up to a dose level of 2000mg /kg body weight of the animal.

9. SUMMARY

The ingredients of Poonaga Parpam were purified and the drug was prepared according to the process mentioned in Anuboga vaidhya navaneetham (Part – 3, Pg.No. 117, Second Edition - 2002, Hakim P. Mohamed Abdulla Sahib), and it was selected for evaluating the toxic effects and mortality when given in short and long duration. The aim of this study is to evaluate the safety of the drug Poonaga Parpam by administering it to Wistar albino rats at various dose levels.

In review of literature, the ingredients of poonaga Parpam were discussed in depth with a special attention paid to their medicinal uses and toxicological aspects.

The ingredients of Poonaga Parpam are poonagam and madhulai. The poonagam were purchased from Sankarankovil and madhulai collected from Tirunelveli.

The raw samples were taken purification and test medicine was prepared, asper the method narrated in the literature.

Biochemical analysis of Poonaga Parpam indicated the presence of calcium, sulphate, chloride, ferrous iron and phosphate. Heavy toxic metals such as lead, mercury, arsenic, and zinc were absent.

Phytochemical analysis of poonaga Parpam shows the presence of carbohydrates, glycosides and tannins.

FTIR study of Poonaga Parpam shows the presene of functional groups such as Halo compounds, alkyl halides, aliphatic amines, nitro compounds, carbon dioxide and alkanes

Scanned Electron Microscope study of end product shows that the particles were stabilized and have irregular morphology. The particles were distributed in range 4µ and the size is below 100 µm

The Acute toxicity study was conducted to know single dose toxicity of POONAGA PARPAM on female Wistar Albino Rats. The study was conducted using 15 female Wistar Albino Rats. The female animals were selected for study of 6 weeks old with weight range of within ±20% of mean body weight at the time of randomization. The groups were numbered as group I, II, III, IV and V and dose with control, 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg of POONAGA PARPAM. The drug was administered by oral route as single dose and observed for 14 days. Daily

the animals were observed for clinical signs and mortality. Body weight of animals was recorded once in a week.

There were no physical and general behavioral changes observed in wistar albino rats of 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg to rats during 14 days. Body weight of all animals did not reveal any significant change as compared to control group.

Food consumption of all group animals was normal

Mortality was not observed in all treated groups.

In Sub-acute toxicity study the animals were selected randomly grouped into 3 different groups containing minimum 6 animals (3 male, 3 female in each group) . The groups were numbered as group I, II, and dose with control, 50mg/kg (low dose), 100mg/kg (High dose), of POONAGA PARPAM. The POONAGA PARPAM was administered as single dose for 28 days and all animals were observed daily once. These observations were also performed on week ends.

The observations included clinical signs of toxicity, food intake, water intake, body weight. No signs of toxicity were observed. There was no significant changes in food intake, water intake and body weight. No mortality occurred till the last day of the study.

The blood samples were used to evaluate Hematological parameters (like RBC, WBC, HB,DC) and evaluate biochemical parameters (like SGOT, SGPT, ALP, UREA and CREATININE). No changes in haematological parameters and biochemical parameters.

On completion of the 28^{th} day of drug administration, Wistar Albino Rats were sacrificed. In macroscopic examination the Heart, Kidneys and Liver organs were weighed. The organs were normal when compared with control group. Histopathological examination revealed normal architecture in comparison with control and drug treated animal.

10. CONCLUSION

Poonaga parpam was studied for its acute and sub-acue toxicity effect using laboratory animals. In acute toxicity study, Poonaga parpam did not produce any specific toxicity or mortality even at the dose of 2000mg/kg in wister albino rats.So No - Observed – Adverse – Effect – Level (NOAEL) of Poonaga parpam is 2000mg/kg body weight of animal. In sub-acute toxicity study, 50 and 100mg/kg of Poonaga parpam was used and it was administered once daily for 28 days through oral route. In conclusion Poonaga Parpam can be considered safe, as it did not cause either any lethality or adverse changes with general behavior of rats and also there were no observable detrimental effects (50mg/kg, 100mg/kg) over a period of 28 days. It is concluded that the Poonaga Parpam is relatively safe when administered orally in human for long administration upto the dose 100mg/kg.

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